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PLANKTON ENERGETICS OF RARITAN BAY

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ABSTRACT

Plankton production in Raritan Bay is described based on total chlorophyll data and two series of 24-hr dark and light bottle differential oxygen experiments. Maximum chlorophyll (γ) recorded was 663 μg L^{-1} in a bloom of *Massartia rotundata*. Utility of pigment data in estimating productivity or biomass is regarded as dubious: i) 17.3×10^6 chains of *Skeletonema costatum* once corresponded to only trace quantities of chlorophyll; ii) production occurred several times in absence of detectable chlorophyll.

In the first (1957) series of experiments, gross production (π) at 1 ft ranged from 4.8 to 531.6 μg O_2 L^{-1} hr^{-1}. Ratios of $\pi\gamma^{-1}$ (when $\gamma > 0$) varied from –0.4 to 14.1 μg O_2 L^{-1} hr^{-1} μg^{-1}. Respiration (ρ) at 1 ft ranged from –49.1 to 230.8 μg O_2 L^{-1} hr^{-1}, and net production ($\pi - \rho$) from –24.7 to 483.0.

In the second (1959) series at a station 15 ft deep, chlorophyll in the whole water column varied from 14.68 to 173.14 μg cm^{-2} (mean 50.75). The range of π was 1.65–13.90, of ρ 1.42–24.51, and of ($\pi - \rho$) –14.63 to 4.53 cal cm^{-2} day^{-1}. Compensation depths ranged from 0 to 6.3 ft (mean 3.0); compensation points varied from 37.1 to 315.7 cal cm^{-2} day^{-1} (mean 169.3). A model is developed which demonstrates that *compensation* is the critical variable in water mass metabolism, and that determination of this variable is the main reason for experimentation. Gross production per unit light intensity (I) at a given depth z, $(\pi I^{-1})_z$, ranged 0.3–828.6 μg O_2 L^{-1} cal^{-1} cm^{-2}. Chlorophyll activity, $(\pi I^{-1} \gamma^{-1})_z$, varied from 0.03 to 6.62 μg O_2 L^{-1} cal^{-1} cm^{-2} μg^{-1}. Total production in the water column relative to incident radiation, $\pi_{0 \to z} I_0^{-1}$, ranged from 0.17 to 1.78% (mean 0.88%). Production efficiency relative to amount of light in the water column, $(\pi I^{-1})_{0 \to z}$, was 0.96–28.71 cal $kcal^{-1}$ (mean 9.07 or 0.907%). Thermodynamic cost of energy procurement varied from 0.54 to 2.58 cal cal^{-1} (mean 1.48), indicating a net energy loss during the study. It is concluded that even if this indicated dystrophy is widespread in the upper estuary, sufficient energy is probably introduced from Lower Bay in the form of standing planktonic biomass to bring the system into positive energy balance.

INTRODUCTION

The primary fixation and subsequent utilization of energy by biotic communities is perhaps the most fundamental action performed. Although energy flow pathways through communities are complex (Lindeman 1942; Clarke 1946; MacFadyen 1948; Odum and Odum 1955; Odum 1956, 1957; Teal 1957, 1959; Patten 1959*a*), the overall process is simple. The ecosystem, itself an aggregation of energy states, continually sacrifices some of its energy to the second law of thermodynamics. Consequently, to preserve its integrity it must procure more energy from the environment. When rate of accrual equals rate of attrition, steady state prevails. When unequal, the system grows or declines, depending upon the direction of the inequality.

This paper is concerned with energy relationships in Raritan Bay plankton. It de-

scribes the annual distribution of chlorophyll in the estuary, and reports the results of two series of light and dark bottle production experiments.

METHODS

Total chlorophyll (γ) was determined by millipore (type HA) filtering a sample, grinding filtrate and filter with sand in 90% acetone containing $MgCO_3$, and then extracting in the dark for at least 12 hr. No chlorophyll was lost under these conditions (see Creitz and Richards 1955, Ryther and Yentsch 1957). The extract was subsequently Seitz-filtered to remove sand and undissolved millipore filter fragments, after which absorption of light passed through a #66 filter was determined with a Lumetron colorimeter. Percentage transmittance was converted to optical density, and then to total chlorophyll using a standard curve prepared from pure chlorophyll *a*. The values obtained were corrected for original volume of water filtered, and total chlorophyll expressed as $\mu g\ L^{-1}$.

Two series of dark and light bottle experiments were performed, one in 1957 and the other in 1959. The series in 1957 was a pilot study, with emphasis on developing a feasible field technique. A more elaborate experimental design was employed in 1959.

In the 1957 experiments paired B.O.D. or citrate bottles were filled with duplicate samples from different depths, and suspended *in situ* for as close to 24 hr as possible, then retrieved and fixed for oxygen titration by the unmodified Winkler method. Gross production (π), community respiration (ρ), and net community production ($\pi - \rho$) were computed from the initial and final dissolved oxygen concentrations. When desirable, outputs in mg $O_2\ L^{-1}$ were converted to cal cm^{-2} based on a $PQ(+O_2/-CO_2)$ of 1.25 (Ryther 1956). When chlorophyll was assayed, $\pi\gamma^{-1}$ ratios were calculated.

The 1959 series was restricted to one station on the south edge of the ship channel off Princess Bay, Staten Island (Fig. 1). Mean low water depth at the station was about 15 ft. "Master" samples were collected from 1, 5, and 9 ft in an inert container and mixed thoroughly to assure identical communities in replicate bottles. Samples from *each* collection depth were suspended at 1, 5, and 9 ft to compare their behavior under variable conditions of light (principally) and temperature. Thus, each experiment consisted of 9 light bottles and 9 dark bottles: L1-1, L1-5, L1-9, L5-1, ..., L9-9; D1-1, ..., D9-9, where the number to the left represents collection depth and that to the right depth of suspension. Each experiment was initiated as close to 1200 hr (EST) as possible, and terminated 24 hr later.

Incident solar radiation (I_0) was obtained with an Eppley 10-junction pyrheliometer situated less than 10 miles from the site. Extinction coefficients (η) were determined with the Lumetron colorimeter using a neutral filter.

CHLOROPHYLL

Chlorophyll assay has developed into one of the important techniques for estimating primary production (Ryther 1956). The method is based on the well-documented constancy of photosynthesis and chlorophyll concentration at any given light intensity (Emerson 1929, Emerson and Arnold 1932, Fleischer 1935, Gaffron and Wohl 1936, Emerson, *et al.* 1940, Manning and Juday 1941, Gessner 1949, Blaauw–Jansen, *et al.* 1950, Edmondson 1955, and Ryther 1956). This constancy is surprising, really, when one considers: i) the known loss of efficiency at high concentrations brought about by high population densities (Tamiya, *et al.* 1953, McQuate 1956, Verduin 1959), ii) the occurrence of inactive chlorophyll (Smith 1954), iii) the association of large quantities of chlorophyll and its degradation products with non-living detritus (Gillbricht 1951, Krey 1952) and sediments (Vallentyne 1955, Vallentyne and Bidwell 1956, Vallentyne and Craston 1957), and iv) the seasonal variations in ratios of chlorophyll to phytoplankton organic matter (Harris and Riley 1956). Ryther and Yentsch (1957) have developed an empiri-

TABLE 1. *Annual distribution of surface chlorophyll concentration at stations 1–6. The units are μg L^{-1}; t signifies trace quantities (less than 4 μg L^{-1})*

Date	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
July 11, 1957	35.2	–	35.5	52.8	–	53.9
18	43.6	62.3	104.2	75.0	–	–
26	20.8	61.3	59.6	49.8	71.3	61.0
Aug 1	48.4	45.6	81.5	67.1	41.3	76.5
8	0.0	0.0	0.0	7.8	0.0	25.6
15	37.5	24.5	22.7	22.7	97.0	21.9
21	58.5	34.7	43.0	55.0	89.1	65.8
28	–	–	–	–	57.9	49.6
29	33.4	44.6	64.0	43.7	64.7	62.6
Sept 9	–	–	28.6	7.2	–	–
12	18.9	7.4	6.8	13.4	20.5	13.4
23	15.0	12.4	19.5	8.8	25.7	10.9
Oct 8	9.4	17.4	20.5	20.2	19.2	9.5
15	–	–	2.1	–	–	–
28	–	–	0.0	–	–	–
29	*t*	0.0	*t*	*t*	7.9	*t*
Nov 19	0.0	0.0	0.0	0.0	0.0	0.0
Dec 6	0.0	0.0	0.0	0.0	0.0	–
17	–	–	0.0	–	0.0	–
20	0.0	*t*	–	0.0	–	–
Jan 20, 1958	9.7	*t*	*t*	10.0	6.4	*t*
Feb 6	11.3	*t*	*t*	8.4	*t*	*t*
Mar 1	9.9	*t*	*t*	8.5	*t*	7.3
11	*t*	4.8	4.8	4.6	4.6	4.8
Apr 10	*t*	*t*	0.0	4.9	5.3	0.0
24	26.3	88.5	76.0	94.4	61.5	61.8
May 10	0.0	0.0	0.0	0.0	0.0	0.0
30	9.5	9.8	10.1	17.2	25.8	18.3
June 23	43.8	22.3	38.2	21.6	34.6	20.4
July 7	33.2	25.4	27.7	162.8	57.7	46.6
24	27.5	36.6	39.3	29.8	40.0	39.8
Aug 11	34.7	79.2	56.8	95.7	87.6	80.6
21	8.4	8.2	13.6	22.7	15.0	22.7

cal equation for estimating production from chlorophyll concentration, solar radiation, and visible light extinction based on the assumption of a parallel relationship. Later (1958, Fig. 2) they give data for the continental shelf region southeast of Raritan Bay showing almost a 25-fold variability in production per unit chlorophyll at optimum (*sic*) light intensities. Verduin (1959) has recently indicated that winter phytoplankton communities are comparably productive to those of summer (which presumably contained more chlorophyll per unit biomass) under light intensities only 1/40 as great. Thus, it would seem that further study is necessary before the efficacy of chlorophyll as an index to productivity becomes unequivocal.

The seasonal aspect of chlorophyll distribution in Raritan Bay is confounded with a daily periodicity (Yentsch and Ryther 1957), making the hour of sampling a consideration. In general, highest chlorophyll concentrations prevailed during the summer, diminishing in September and October to virtual and literal absence in late October, November, and December (Table 1). Traces of chlorophyll began to appear in January, and low levels prevailed throughout the early spring to mid-April. On 24 April, 1958, at the height of the vernal diatom flowering, high chlorophyll levels were recorded throughout the estuary. After the bloom declined, chlorophyll was absent for a period, then gradually increased to late summer maxima.

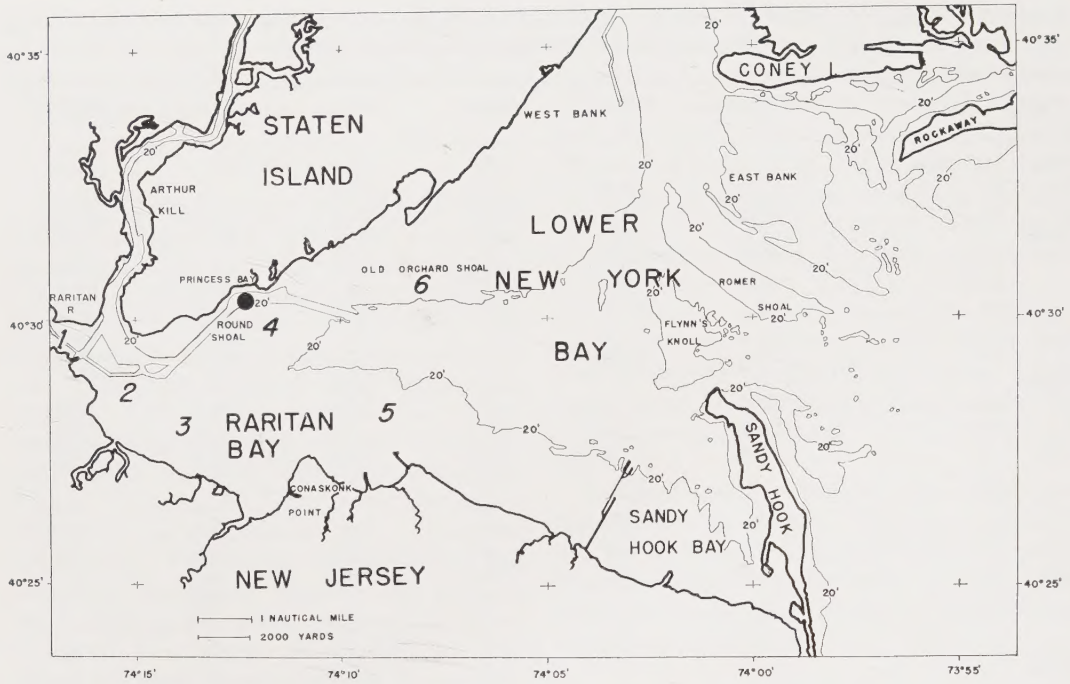

FIG. 1. Raritan Bay, showing sampling stations (1–6) and dark and light bottle station (●).

Chlorophyll concentrations did not always correlate well with phytoplankton population levels in the estuary. For example, the low autumnal levels did not reflect adequately the high concentrations of colorless chrysoflagellates (especially *Oxyrrhis marina*) dominant during this period. Also, on one occasion during the spring bloom, dominated by *Skeletonema costatum*, 17.3×10^6 units of *Skeletonema* per liter yielded only trace quantities of pigment.

Lowest chlorophyll was generally recorded at stations 1 and 2 at the head of the bay (Fig. 1). The highest levels of summer, 1957, occurred at stations 3 and 5, but in summer, 1958, greatest concentrations were at stations 4, 5, and 6. Red water was encountered in July, 1957; in late May, 1958; and again in June and July, 1959. The principal red tide organism in 1959 was *Massartia rotundata*. Other forms associated as minor constituents were *Eutreptia* sp., *Nannochloris atomus*, *Pyramimonas* sp., *Peridinium trochoideum*, *Gymnodinium* sp., *Skeletonema costatum*, and tintinnids. Chlorophyll levels recorded in the 1957 red tides ranged from 75 to 105 μg L^{-1}. The highest concentration recorded in the bay prior to the summer of 1959 was 162.8 μg L^{-1} at station 4 on 7 July, 1958. Strangely, the water on this occasion did not appear red, possibly because the principal organism was *Nannochloris* (40.6×10^7 units L^{-1}) instead of *Massartia*. The value of 162.8 μg L^{-1} is considerably less than maxima recorded by Riley (1956) of 314 μg L^{-1} in Long Island Sound, and by Pomeroy, *et al.* (1956) of 360 μg L^{-1} in Delaware Bay in 1955. However, during the red water period of 1959, a surface sample was collected on 30 June from center-bay northeast of Conaskonk Pt. which assayed 663 μg L^{-1}. High winds on 1 July completely dispersed this aggregation (*Massartia*) so that no evidence of it remained on 3 July.

Chlorophyll was not usually vertically uniform throughout the water column. Table 2 presents data for some profiles obtained during summer and autumn, 1957. Highest chlorophyll concentration was gen-

TABLE 2. *Vertical distribution of chlorophyll on several dates during summer and autumn, 1957. Chlorophyll concentrations, γ, are given in $\mu g\ L^{-1}$; differentials, $\Delta\gamma$, are also indicated*

Depth (feet)	Station 2 γ	$\Delta\gamma$	Station 3 γ	$\Delta\gamma$	Station 4 γ	$\Delta\gamma$	Station 5 γ	$\Delta\gamma$	Station 6 γ	$\Delta\gamma$
	(Sept 9)		(Sept 9)		(Sept 9)		(Aug 28)		(Aug 28)	
0	–		–				57.9		49.6	
								−7.8		5.6
1	11.7		27.2		8.6		50.1		55.2	
		25.4		−0.4		8.8		−2.7		0.3
3	37.1		26.8		17.4		47.4		55.5	
		−1.3		−8.7		−9.6		−0.2		0.3
5	35.8		18.1		7.8		47.2		55.8	
								−11.0		4.9
7							36.2		60.7	
								10.3		−4.6
11							46.5		56.1	
								−10.1		0.1
15							36.3		50.2	
								0.7		−2.6
17							37.0		–	
19									47.6	
	(Sept 23)		(Sept 23)		(Sept 23)					
0	–		25.7		–					
				1.1						
1	12.4		26.8		10.9					
		−1.5		0.0		−1.6				
3	10.9		26.8		9.3					
		−0.9		−0.5		1.2				
5	10.0		26.3		10.5					
	(Oct 28)				(Oct 28)					
1	trace				0.0					
3	trace				0.0					
5	0.0				0.0					

erally several feet beneath the surface. Vertical homogeneity did occur late in September, associated with the more complete mixing attending breakdown of summer stratification, and also with dominance by non-motile diatoms at that time. Further data on this aspect are presented later which show that motile organisms with highly active (photosynthetically) chlorophyll can maintain a vertical gradient of chlorophyll activity, although not necessarily of concentration.

BOTTLE EXPERIMENTS—1957

Criticisms of the Gaarder and Gran (1927) dark and light bottle technique may be grouped into four categories: i) stagnation of the closed cultures with time (Ryther 1956, Verduin 1956); ii) tendency to overestimate π and ρ because of a) differential development of bacteria in dark and light bottles due to inhibition by light (Steemann Nielsen 1952) or by photosynthesizing phytoplankton (Steemann Nielsen 1955), and b) inordinate bacterial growth made possible by increased surface area of containers (Pratt and Berkson 1959); iii) tendency to underestimate π and ρ with consequent under- or overestimation of $(\pi - \rho)$ due to settling of organisms (Verduin, *et al.* 1959); iv) tendency to underestimate light bottle respiration, hence π, because of differential biomass changes in light vs. dark. Of these criticisms, the

last is most basic because it is insuperably bound to the very thing the experiment endeavors to measure. The other three factors are potentially subject to empirical control.

Ryther and Yentsch (1958), studying the annual productivity of the continental shelf waters off New York, estimated an average 0.2–1.0 g C m^{-2} day^{-1} gross production. This is equivalent to 22.2–111.2 μg O_2 L^{-1} hr^{-1}, assuming a photosynthetic quotient ($+O_2/-CO_2$) of unity. In the 1957 series of dark and light bottle experiments in Raritan Bay, photosynthesis at 1 ft ranged from 4.8 to 531.6 μg O_2 L^{-1} hr^{-1} (Table 3). Gross production generally decreased with depth due to reduced light, but not in every case since more chlorophyll may have been present in some of the deeper samples. Production per unit chlorophyll, $\pi\gamma^{-1}$, was computed whenever possible. These ratios varied considerably from date to date and station to station, although highest values always occurred at the surface. On several occasions positive production was measured in absence of detectable chlorophyll (denoted by $\pi\gamma^{-1} = \infty$ in Table 3). When chlorophyll was present, production per μg ranged from –0.39 to 14.09 μg O_2 L^{-1} hr^{-1}, the negative value having been obtained at 7 ft. Respiration rate ranged from –49.1 to 230.8 μg O_2 L^{-1} hr^{-1} at 1 ft. The several negative values obtained for respiration suggest either light leakage into the dark bottles or non-photosynthetic production. The $\pi\gamma^{-1}$ ratios of ∞ also indicate the possibility of chemoautotrophic mechanisms. Net community production at 1 ft depth ranged from –24.7 to 483.0 μg O_2 L^{-1} hr^{-1}, the maximum value being about four times the maximum offshore gross production recorded by Ryther and Yentsch (1958).

BOTTLE EXPERIMENTS—1959

Hydrography

This series comprised 11 experiments, one each week between 16 June and 25 August. The hydrographic data are summarized in Table 4.

Salinity varied from 24.45 to 26.67‰. Surface water was generally somewhat less saline than mid-depths, and deeper waters slightly more saline. The maximum vertical gradient encountered was only 0.81‰, the mean 0.44‰, and the minimum 0.03‰, indicating a reasonably well-mixed water column.

Temperature varied from 18.00°C on 16 June to 26.32 on 17 August. Maximum vertical gradient was 2.82°C on 8 July, minimum 0.06 on 24 August, and the mean gradient was 0.99°C. Temperature was always greater at the surface, except on 15 July when an inverse gradient was observed, and on 24 August when some surface cooling was in evidence. These temperature patterns also indicate a fairly homogeneous water column.

Secchi disc data proved very unreliable, and showed only little relationship to extinction coefficients obtained by measuring transmittance through a neutral filter. Minimum depth at which the disc disappeared was 45 in. on 16 June, maximum was 82 in. on 17 August, and the mean for the summer was 62 in.

Fate of Solar Radiation

Insolation, I_0, impinges on a water surface directly from the sun and indirectly from the sky. Part of this radiation, I_R, is reflected from the surface or back-scattered. The total loss to I_R is variable, but will be regarded here as 6% of I_0 (see Hutchinson 1957). The remainder, I_A, is transmitted ($I_{0\to z}$) and absorbed (I_α). The absorption rate is expressed by the extinction coefficient:

$$\eta = z^{-1} \ln (I_0 I_z^{-1}) ,$$

where I_z is light intensity at depth z. Calculated values of η were used to compute I_z at several depths, and these figures were then graphed. A typical graph (for expt. I) is provided in Fig. 2, the curve being drawn through the points I_A, $I_{z=1}$, I_5, and I_9. The following areas may be distinguished:

1) ACDF, the potential transmission, I_A, to depth z in absence of an absorbing medium.

TABLE 3. *Summary of 1957 dark and light bottle experiments. Chlorophyll concentration, γ, is in $\mu g\ L^{-1}$; $\pi\gamma^{-1}$ ratios are given wherever the data permitted their calculation; values of ∞ indicate production in absence of detectable chlorophyll*

Station	Depth (ft)	Time (EST) Start	End	Duration (hr)	π	ρ	$\pi - \rho$	γ	$\pi\gamma^{-1}$
				JULY 18–19					
2	1	1310	1245	23.58	–	–	–	62.3	–
	5				89.5	138.3	–48.8	–	–
	7				77.6	64.5	13.1	–	–
3	1	1400	1320	23.67	–	–	83.2	104.2	–
	4				–	–	–171.5	–	–
	7				–	23.7	–	–	–
4	1	1450	1415	23.58	149.7	75.9	73.8	75.0	2.00
	4				38.2	92.0	–53.9	–	–
	7				48.3	337.2	–288.8	–	–
				AUG. 7–8					
2	1	1110	0915	22.10	108.6	25.8	82.8	0.0	∞
	3				46.2	24.9	21.3	0.0	∞
	5				24.4	34.4	–10.0	0.0	∞
5	1	1155	1140	23.67	271.2	95.9	175.3	0.0	∞
	5				81.5	68.0	13.5	–	–
	9				0.0	52.4	–52.4	13.7	0.00
	14				–	–	–	–	–
6	1	1235	1250	24.25	261.4	103.5	157.9	25.6	10.21
	7				113.0	79.2	33.8	–	–
	15				17.5	52.0	–34.6	12.5	1.40
	19				–	–	–	–	–
				AUG. 14–15					
2	1	1045	1350	27.10	7.4	–24.7	32.1	24.5	0.30
	3				38.4	23.6	14.8	–	–
	7				–4.8	15.5	–20.3	12.4	–0.39
				AUG. 20–21					
2	1	0945	1315	27.50	489.0	132.0	357.1	34.7	14.09
	3				293.1	175.3	117.8	–	–
	7				26.2	98.9	–72.7	–	–
	11				–	–	–	34.0	–
				AUG. 28–29					
2	1	1430	1420	23.83	531.6	48.7	483.0	44.6	11.92
	3				139.3	57.5	81.8	–	–
	5				26.9	42.0	–15.1	34.7	0.78
5	1	1000	0950	23.83	148.6	–49.1	197.7	50.1	2.97
	5				–	–	–21.8	47.2	–
	9				–	–	–43.6	37.4	–
6	1	1230	1100	22.50	183.6	16.4	167.1	55.2	3.33
	5				145.8	61.3	84.4	55.8	2.61
	9				40.0	58.7	–18.7	–	–
				SEPT. 9–10					
2	1	1500	1500	24.00	6.7	21.3	–14.6	11.7	0.57
	3				–	13.3	–	37.1	–
	5				–	–	–20.8	35.8	–
between	1	1155	1535	27.67	23.5	10.1	13.4	27.2	0.86
3 and 5	3				2.9	–15.2	–12.3	26.8	0.11
	5				17.0	20.6	–3.6	18.1	0.94
between	1	1255	1600	27.10	–	–	–24.7	8.6	–
4 and 6	3				–	–	–22.9	17.4	–
	5				–	–	–	7.8	–

TABLE 3. (Continued)

Station	Depth (ft)	Time (EST) Start	End	Duration (hr)	π	ρ	$\pi - \rho$	γ	$\pi\gamma^{-1}$
				SEPT. 23–24					
2	1	1230	1430	26.00	–	–	230.8	12.4	–
	3				125.8	28.8	96.9	10.9	11.54
	5				30.8	28.8	1.9	10.0	3.08
between	1	1345	1235	22.83	–	–	42.0	10.9	–
4 and 6	3				23.2	9.2	14.0	9.3	2.51
	5				11.8	18.8	–7.0	10.5	1.12
				OCT. 28–29					
2	1	1400	1630	26.50	10.2	10.2	0.0	0.0	∞
	3				20.0	12.1	7.9	0.0	∞
	5				12.1	6.0	6.0	0.0	∞
4	1	1500	1345	22.75	4.8	14.5	–9.7	0.0	∞
	3				26.4	44.8	–18.5	0.0	∞
	5				8.4	18.9	–9.7	0.0	∞

2) ABEF, the potential transmission not realized at depth z due to absorption.

3) BCDE, the amount of light remaining at depth z.

4) ACD′E′, the total light, $I_{0\to z}$, transmitted to depth z where

$$I_{0\to z} = I_0 \int_0^z e^{-\eta z}\, dz$$

$$= I_0 \eta^{-1}(1 - e^{-\eta z}) .$$

5) ABE′, the fraction, $I'_{0\to z}$, of the total transmitted light absorbed.

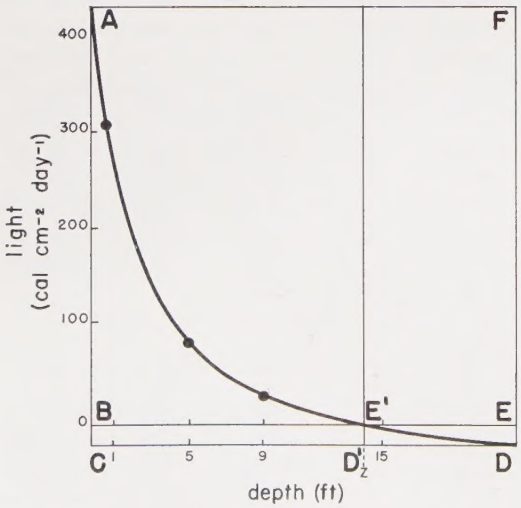

FIG. 2. Diagram of fate of solar radiation in a water column (description in text).

6) AE′EF, the total light, I_α, not transmitted to depth z due to absorption ($I_\alpha = I_A - I_{0\to z}$). In practice $I'_{0\to z}$ and $I_{0\to z}$ were always equal at this station since light was always completely extinguished at 15 ft. The quantity $I_{0\to z}$ is the total energy available for photosynthesis in the water column. Table 5 summarizes the data on I_0, I_R, I_A, $I_{0\to z}$, $I_{0\to z} I_0^{-1}$, and η obtained for each of the 11 experiments. I_0 ranged from 277.4 to 953.6 cal cm^{-2} day^{-1}; $I_{0\to z}$ varied from 309.5 to 1,346.0 cal cm^{-2} day^{-1}.

Chlorophyll Distribution

Initial chlorophyll concentrations, γ, at the beginning of each experiment are reported in Table 6 for each experimental depth. The range at 1 ft was 5.97–135.42 μg L^{-1}, at 5 ft 10.65–106.25 μg L^{-1}, and at 9 ft 10.42–56.70 μg L^{-1}. The vertical distribution is shown to be not uniform. Highest values generally occurred at the surface (8 out of 11 times), and lowest either at 9 ft (6 out of 11) or 5 ft (4 out of 11). Gradients were generally slight at low concentration levels (mean 6.58 μg L^{-1}), but steeper when high concentrations were present (experiments IV and VII, mean 69.93). The maintenance of high γ concentrations in the surface layers may be due in part to motility of the organisms. The principal organism at 1 ft in experiment IV was *Massartia*, associated

TABLE 4. *Hydrography of the sampling station opposite Princess Bay during the summer of 1959*

Experiment	Dates	Salinity, ‰			Secchi disc (in.)	Temperature, °C						
		1 ft	5 ft	9 ft		Surface	1	3	5	7	9	Bottom
I	Jun 16	–	–	–	45	18.00	–	–	–	–	–	–
	17					–	–	–	–	–	–	–
II	Jun 23	26.47	26.52	26.67	49	19.00	19.00	19.00	18.80	18.55	18.35	–
	24					18.85	18.70	18.65	18.58	18.50	18.45	–
III	Jul 2	26.34	25.80	25.92	61	23.00	22.95	22.93	22.90	22.90	23.00	22.75
	3					23.22	22.98	22.80	22.68	22.62	23.30	22.45
IV	Jul 8	25.04	25.23	25.48	59	25.82	25.40	24.47	23.60	23.60	23.40	23.00
	9					25.15	24.70	24.67	24.23	24.05	23.83	23.45
V	Jul 15	25.24	25.50	25.57	75	23.40	23.48	23.57	23.52	23.50	23.50	23.45
	16					23.40	23.40	23.35	23.30	23.29	23.29	23.24
VI	Jul 20	24.92	25.10	25.60	80	–	–	–	–	–	–	–
	21					25.64	24.84	24.38	24.13	24.10	24.07	23.90
VII	Jul 29	25.60	25.82	26.13	39	25.72	25.50	25.38	25.27	25.05	24.85	23.50
	30					–	–	–	–	–	–	–
VIII	Aug 3	24.87	25.73	25.68	62	25.80	–	–	–	–	–	–
	4					–	–	–	–	–	–	–
IX	Aug 10	24.45	24.61	24.69	62	23.44	23.08	23.00	22.87	22.85	22.56	22.19
	11					–	–	–	–	–	–	–
X	Aug 17	24.68	25.14	25.36	82	26.32	25.97	25.15	25.11	25.10	25.10	25.10
	18					25.70	25.51	25.30	25.25	25.22	25.21	25.21
XI	Aug 24	26.27	26.24	26.27	63	23.34	23.40	23.40	23.40	23.40	23.40	23.40
	25					24.10	24.10	24.08	24.00	23.82	23.50	23.20

with *Eutreptia* and *Pyramimonas*. At 5 ft and 9 ft, however, *Nannochloris* and *Skeletonema*, both non-motile, were dominant, and *Massartia* was entirely absent. In this experiment there was no vertical salinity gradient, but there was a considerable thermal gradient, with the warmer water sharply restricted to the surface (Table 4). In experiment VII, the whole water column was dominated by *Nannochloris* and *Skeletonema*, including the surface, although *Massartia* was the third most important species in cell numbers in the upper 1 ft, was rare at 5 ft and absent at 9 ft. In view of what has previously been said about the low chlorophyll content of *Skeletonema*, it seems likely that most of the pigment on this occasion was contributed by *Nannochloris* and *Massartia*, and the gradient resulted from ability of motile *Massartia* to maintain itself at the surface. No salinity gradient existed in experiment VII, but the temperature gradient was considerable,

TABLE 5. *Radiation parameters for each experiment performed during summer, 1959. Values are in cal cm^{-2} day^{-1}. Extinction coefficients (η) are also provided*

Experiment	I_0	I_R	I_A	$I_{0\to z}$	η
I	457.5	27.5	430.0	567.4	1.16
II	899.0	53.9	845.1	801.9	1.65
III	848.7	50.9	797.8	642.4	1.85
IV	953.6	57.2	896.4	811.1	1.75
V	277.4	16.6	260.8	309.5	1.38
VI	440.7	26.4	414.3	682.1	0.93
VII	551.6	33.1	518.5	342.4	2.43
VIII	944.4	56.7	887.7	1,346.0	1.05
IX	781.3	46.9	734.4	1,083.1	1.05
X	428.8	25.7	403.1	652.0	0.99
XI	331.2	19.9	311.3	572.0	0.81

TABLE 6. *Chlorophyll concentrations, γ_z, at each sample depth, in μg L^{-1}*

Experiment	Sample depths		
	1 ft	5 ft	9 ft
I	15.70	16.17	16.36
II	27.59	21.18	20.55
III	23.03	22.96	22.10
IV	96.84	43.95	35.70
V	17.66	27.88	11.36
VI	5.97	10.73	10.42
VII	135.42	106.25	56.70
VIII	28.27	15.27	16.20
IX	23.21	16.85	20.24
X	21.74	17.66	14.20
XI	13.49	10.65	11.01

TABLE 7. *Vertical temperature profile 150 yards NNE of sta. 3, 19 July, 1957*

Depth (in.)	°C
1	28.5
3	28.8
6	27.6
12	26.2
24	25.4
150	23.6

with warmer water penetrating to a depth of 7 ft (Table 4). The effect of reradiation of long-wave radiation by surface plankton blooms on water temperature is indicated in Table 7, where a gradient of 3°C in 2 ft of depth is noted. The resulting tendency to some degree of thermally-induced stability in the water column is doubtless another factor in the maintenance of red water communities at the surface.

Total chlorophyll in the whole water column was obtained by planimetric integration of the curves connecting γ_z ($z = 1$, 5, and 9 ft), and extrapolating to $z = 0$ and $z = 15$. These values are summarized in Table 8, which shows a range of $\gamma_{0\to z}$ from 14.68 to 173.14 μg cm^{-2}.

Production Parameters

Table 9 summarizes results for *in situ* π, ρ, and $(\pi - \rho)$. Data for depth combinations other than 1-1, 5-5, and 9-9 will be discussed later. As shown in Table 9, gross production at 1 ft ranged from 24.2 to 625.0, at 5 ft from 3.3 to 48.3, and at 9 ft from 0.4 to 19.2 μg O_2 L^{-1} hr^{-1}. The values of π_z ($z = 1$-1, 5-5, and 9-9) for each date were plotted, and the area under the curves determined planimetrically to obtain $\pi_{0\to z}$, the total gross production in the whole water column. Values of $\pi_{0\to z}$, converted from mg O_2 L^{-1} to cal cm^{-2} day^{-1}, are given in Table 8. Community respiration, ρ_z, was 15.8–289.6 μg O_2 L^{-1} hr^{-1} at 1 ft, 11.3–217.9 μg O_2 L^{-1} hr^{-1} at 5 ft, and 11.3–89.6 μg L^{-1} hr^{-1} at 9 ft. As with π, the values of ρ_z were plotted, a curve drawn through the points, and the area under the curve estimated planimetrically to obtain total community respiration in the water column. These values are also presented in cal cm^{-2} day^{-1} in Table 8. Community net production, $(\pi - \rho)_z$, also appears in Table 9. The values ranged from –6.7 to 530.8 μg O_2 L^{-1} hr^{-1} at 1 ft, –192.5 to 14.2 μg O_2 L^{-1} hr^{-1} at 5 ft, and –81.3 to –4.6 μg O_2 L^{-1} hr^{-1} at 9 ft, indicating a shallow trophogenic zone, no more than several feet in depth on the average. Even when surface gross production was substantial, as in experiments IV and VII (625 and 352 μg O_2 L^{-1} hr^{-1} at 1 ft, respectively), the net product at 5 ft and below was negative. Thus, it appears that the red water organisms involved, in order to maintain a favor-

TABLE 8. *Integrated values of the variables* $y = I, \gamma, \pi, \rho$, *and* $(\pi - \rho)$, *per 24 hr;* i.e., $y_{0\to z} = \int_0^{15} y_z\, dz$.

Radiation and production parameters are in cal cm^{-2} day^{-1}; $\gamma_{0\to z}$ *is in* μg cm^{-2}

Experiment	$I_{0\to z}$	$\gamma_{0\to z}$	$\pi_{0\to z}$	$\rho_{0\to z}$	$(\pi - \rho)_{0\to z}$
I	567.4	33.38	3.47	4.71	–1.37
II	801.9	45.31	8.41	4.57	3.84
III	642.4	45.67	4.53	7.27	–2.74
IV	811.1	100.31	13.90	9.37	4.53
V	309.5	30.27	2.10	4.30	–2.20
VI	682.1	19.71	1.65	1.97	–0.32
VII	342.4	173.14	9.83	24.46	–14.63
VIII	1,346.0	39.27	7.59	8.50	–0.91
IX	1,083.1	14.68	6.08	9.60	–3.52
X	652.0	32.19	5.76	6.49	–0.73
XI	572.0	24.09	0.55	1.42	–0.87
Means		50.75	5.81	7.50	–1.69

TABLE 9. *Values of π_z, ρ_z, and $(\pi-\rho)_z$, in $\mu g\ O_2\ L^{-1}$, obtained in the 1959 experiments*

Experiment	π_z			ρ_z			$(\pi-\rho)_z$		
	1-1	5-5	9-9	1-1	5-5	9-9	1-1	5-5	9-9
I	91.5	19.2	7.7	31.1	21.2	25.4	60.4	−1.9	−17.7
II	233.3	48.3	12.5	45.0	34.2	23.3	188.3	14.2	−10.8
III	120.8	25.4	12.5	45.8	36.3	35.8	65.0	−10.8	−23.3
IV	625.0	42.1	19.2	94.2	79.6	81.3	530.8	−37.5	−62.1
V	51.7	14.2	2.1	25.8	63.8	16.7	25.8	−49.6	−14.6
VI	50.0	3.3	7.1	15.8	11.3	11.7	34.2	−7.9	−4.6
VII	351.7	25.4	8.3	289.6	217.9	89.6	62.1	−192.5	−81.3
VIII	292.1	16.7	5.8	66.3	32.5	44.2	225.8	−15.8	−38.3
IX	210.0	16.3	14.2	67.1	47.1	52.9	142.9	−30.8	−38.8
X	197.5	17.5	1.3	66.3	42.5	37.5	131.3	−25.0	−36.3
XI	24.2	11.7	0.4	30.8	25.8	11.3	−6.7	−14.2	−10.8

able balance between trophogenesis and tropholysis, are inherently restricted to surface layers where light intensity is high. As with π and ρ, $(\pi-\rho)_{0\to z}$ was also determined. These values are given in Table 8 in cal cm^{-2} day^{-1}.

Total chlorophyll in the water column, $\gamma_{0\to z}$, varied from 14.68 to 173.14 μg cm^{-2}, with a mean of 50.75 μg cm^{-2} (Table 8). Two pulses in concentration are indicated, experiments IV and VII. Gross production, $\pi_{0\to z}$, ranged from 0.55 to 13.90 cal cm^{-2} day^{-1}, with a mean of 5.81. Peak photosynthesis occurred in experiments IV and VII corresponding to γ maxima. Community respiration, $\rho_{0\to z}$, varied from 0.31 to 5.35 cal cm^{-2} day^{-1}, with pulses again in experiments IV and VII. In general, a good visual relationship between γ_z and ρ_z was observed in constructing the graphs. The correlation can be made by comparing the ρ_z values in Table 9 with the γ_z values in Table 6. The second mode of ρ is prolonged through experiments VII–X, with a minor pulse in IX not associated with a γ pulse. The significance of this will be discussed later when $(\rho\gamma^{-1})_{0\to z}$ ratios are considered. Net community production, $(\pi-\rho)_{0\to z}$, was negative except in experiments II and IV. The range was from −3.20 to 0.99 cal cm^{-2} day^{-1}, with a mean of −0.37 cal cm^{-2} day^{-1}. Thus, net community production was negative between 16 June and 25 August in spite of consistently substantial photosynthetic rates (mean π_z at 1 ft, 5 ft, and 9 ft, respectively, 204.4, 21.8, and 8.3 μg O_2 L^{-1} hr^{-1}). The reason is that photosynthesis diminishes exponentially with increased depth, whereas respiration remains more or less constant throughout the water column.

Compensation

As indicated above, estuarine productivity is intimately bound up with depth. The best single criterion of the whole dynamic interplay between light, bathymetry, trophogenesis and tropholysis in a water mass is compensation—either compensation depth (c), where photosynthesis equals respiration, or compensation point (I_c), the light intensity at the compensation depth. All the complexity of trophodynamic action devolves to this single variable, as follows.

From Patten (1961), the biomass energy $B(Q)$ at time t in a homogeneous water column is related to that at time 0 by

$$B_t(Q) = B_0(Q) \exp\,[(\pi-\rho)/B(Q)]t\,. \quad (1)$$

If photosynthesis per unit biomass at a given depth may be regarded as a direct function of light intensity:

$$[\pi/B(Q)]_z = \phi\, I_z = \phi\, I_0 e^{-\eta z}\,, \quad (2)$$

then in the whole water column

$$[\pi/B(Q)]_{0\to z} = \int_0^z \phi\, I_0 e^{-\eta z}\, dz$$

$$= \phi\, I_0\, \eta^{-1}(1-e^{-\eta z})\,. \quad (3)$$

Similarly for respiration:

$$[\rho/B(Q)]_z = \lambda \quad (4)$$

and

$$[\rho/B(Q)]_{0\to z} = \int_0^z \lambda\, dz = \lambda z\,. \quad (5)$$

Compensation point, I_c, is related to incident radiation by

$$I_c = I_0 e^{-\eta c}. \quad (6)$$

At the compensation depth, combining (2) and (4),

$$\lambda = \phi\, I_0 e^{-\eta c}$$

which, substituting (6), yields

$$\lambda = \phi\, I_c,$$

or

$$I_c = \lambda \phi^{-1}. \quad (7)$$

Thus, I_c summarizes the relationship between the photosynthesis and respiration coefficients. The *cost* in biomass energy to procure a unit of energy from the biotope may be expressed in terms of four variables by making appropriate substitutions of (7) into (3) and (5) and cancelling:

$$(\rho\pi^{-1})_{0\to z} = \eta z I_c I_0^{-1}(1 - e^{-\eta z})^{-1}. \quad (8)$$

Substitution of (6) into (8) yields

$$(\rho\pi^{-1})_{0\to z} = \eta z e^{-\eta c}(1 - e^{-\eta z})^{-1}. \quad (9)$$

Since η, z, and I_0 can be determined independent of experimentation, it is clear that the whole *raison d'être* of dark and light bottles is to determine I_c (eq. 8) or c (eq. 9). The reason is implicit in equation (7).

Values of c and I_c are provided in Table 10 for each experiment. Compensation depth ranged from nil in the last experiment to 6.3 ft, with a mean of 3.0 ft. Compensation point varied from 37.1 to 315.7 cal cm^{-2} day^{-1}. In experiment XI, respiration exceeded photosynthesis even at the surface so that no compensation point existed.

TABLE 10. *Compensation depths, c (in ft), and compensation points, I_c (in cal cm^{-2} day^{-1})*

Experiment	c	I_c
I	4.6	90.1
II	6.3	37.1
III	3.1	147.4
IV	3.2	174.4
V	1.8	132.3
VI	3.0	188.5
VII	1.6	171.1
VIII	3.4	315.7
IX	2.5	298.2
X	3.8	138.6
XI	0.0	–
Means	3.0	169.3

Efficiencies

The following intensive variables were computed: $(\pi I^{-1})_z$, $(\pi I^{-1}\gamma^{-1})_z$; $\pi_{0\to z} I_0^{-1}$, $(\pi I^{-1})_{0\to z}$, $(\pi I^{-1}\gamma^{-1})_{0\to z}$, $(\rho\gamma^{-1})_{0\to z}$, and $(\rho\pi^{-1})_{0\to z}$.

1. $(\pi I^{-1})_z$: Efficiency of primary production per unit light available at each depth (Table 11) varied from 0.30 to 828.57 $\mu g\ O_2\ L^{-1}\ cal^{-1}\ cm^{-2}$. Two periods of

TABLE 11. *The efficiency $\pi_z I_z^{-1}$ for each sample in the 1959 experiments. The figures are in $\mu g\ O_2\ L^{-1}\ cal^{-1}\ cm^{-2}$*

Experiment	1-1	1-5	1-9	5-1	5-5	5-9	9-1	9-5	9-9
I	7.84	6.65	8.02	3.69	6.65	13.37	4.19	6.78	10.70
II	10.92	27.87	46.81	4.19	16.67	41.49	4.84	19.25	31.91
III	6.36	20.33	62.50	2.85	12.53	152.08	2.15	15.20	62.50
IV	49.44	82.41	136.71	5.21	15.59	62.03	6.64	15.59	58.23
V	7.14	12.90	11.94	4.89	9.97	43.28	1.61	5.28	7.46
VI	3.83	2.53	5.34	1.47	0.78	5.34	1.15	0.49	5.04
VII	33.71	137.50	828.57	13.62	44.85	57.14	15.62	45.59	285.71
VIII	10.85	11.98	14.12	1.64	2.21	3.03	2.06	2.98	2.35
IX	9.46	14.00	11.52	1.88	2.64	6.62	3.98	6.85	8.33
X	15.78	10.11	16.38	5.99	4.52	1.74	5.69	6.02	1.05
XI	2.39	2.22	1.50	1.36	3.11	3.59	1.52	1.56	0.30
Means	14.34	29.86	103.95	4.25	10.87	35.43	4.50	11.42	43.05
Ratios of means	1.00 :	2.08 :	7.25	1.00 :	2.56 :	8.34	1.00 :	2.54 :	9.57

TABLE 12. $\pi_z I_z^{-1} \gamma_z^{-1}$ *at the Princess Bay station, summer, 1959. The units are* $(\mu g\ O_2\ L^{-1})(cal\ cm^{-2})^{-1} \cdot (\mu g\ L^{-1})^{-1}$

Experiment	1-1	1-5	1-9	5-1	5-5	5-9	9-1	9-5	9-9
I	0.50	0.42	0.51	0.23	0.41	0.83	0.26	0.41	0.65
II	0.40	1.01	1.70	0.20	0.79	1.96	0.24	0.94	1.55
III	0.28	0.88	2.71	0.12	0.55	6.62	0.97	0.69	2.83
IV	0.51	0.85	1.41	0.12	0.35	1.41	0.19	0.44	1.63
V	0.40	0.73	0.68	0.18	0.36	1.55	0.14	0.46	0.66
VI	0.64	0.42	0.89	0.14	0.07	0.50	0.11	0.05	0.48
VII	0.25	1.02	6.12	0.13	0.42	0.54	0.28	0.80	5.04
VIII	0.38	0.42	0.50	0.11	0.14	0.20	0.13	0.18	0.15
IX	0.41	0.60	0.50	0.11	0.16	0.39	0.20	0.34	0.41
X	0.73	0.47	0.75	0.34	0.26	0.10	0.40	0.42	0.07
XI	0.18	0.16	0.11	0.13	0.29	0.34	0.14	0.14	0.03
Means	0.42	0.64	1.44	0.16	0.35	1.31	0.28	0.44	1.23
Ratios of means	1.00 :	1.50 :	3.40	1.00 :	2.12 :	8.04	1.00 :	1.60 :	4.44

higher efficiency were observed, in experiments IV and VII, associated with higher than average chlorophyll concentrations (Tables 6 and 8). Extremely low efficiencies were recorded in the last experiment suggesting a loss of productive capacity, and signaling decline of the summer phytoplankton. The summer means and their ratios demonstrate two facts: i) samples collected at the surface (1-1, 1-5, and 1-9) were several-fold more productive per unit light intensity than those collected at 5 ft and 9 ft, indicating greater viability of surface communities. However, ii) production efficiency of a given community per unit light, regardless of collection depth, increased with depth, reflecting the well-known adverse influence of higher light intensities upon photosynthesis. This effect was less pronounced under conditions of low incident light (experiments I, V, VI, X, and XI), and was not observed at all in the last two experiments.

2. $(\pi I^{-1}\gamma^{-1})_z$: The amount of photosynthesis per unit light per unit chlorophyll is a good index to the γ activity of a given bottled community. These data (Table 12) appear to equivocate the thesis of unit chlorophyll activity at a given light intensity. Values ranged from 0.03 to 6.62 $\mu g\ O_2\ L^{-1}\ cal^{-1}\ cm^{-2}$ per $\mu g\ \gamma\ L^{-1}$. The means and their ratios show γ activity to increase with depth, although this effect was manifested principally in the early experiments.

3. $(\pi_{0\to z} I_0^{-1})$: Gross production efficiency in the water column per unit incident radiation at the surface is given in Table 13 in cal $kcal^{-1}$ (1 kcal = 1,000 cal). To convert these figures to percentages move the decimal one place to the left (*e.g.*, the efficiency in experiment I was 0.758%). The values ranged from 1.66 to 17.82 cal $kcal^{-1}$, a 10-fold variability; mean efficiency was 8.81 cal $kcal^{-1}$. These data indicate three peaks in photosynthesis during the summer, experiments IV, VII, and X. The efficiency levels reported are relatively lower than those cited elsewhere in the literature for aquatic ecosystems: various lakes 0.043–0.38% (Clarke 1939), Lake Mendota 0.40% (Juday 1940), Long Island Sound 0.08–0.72% (Riley 1941), Cedar Bog Lake 0.10% (Lindeman 1942), Minnesota pond 0.04% (Dineen 1953), Eniwetok reef 5.80% (Odum and Odum 1955), Silver Springs 1.22% (Odum 1957), and Root Spring 0.20% (Teal 1957). The figures for Raritan Bay are 0.116–1.782%, with a mean of 0.881%.

4. $(\pi I^{-1})_{0\to z}$: This efficiency is more indicative of biological capacity for production than $(\pi_{0\to z} I_0^{-1})$ because it is based on actual light available. The values obtained are also given in Table 13. The range observed was 0.96–28.71 cal $kcal^{-1}$, with a mean of 9.07 (0.907%). So, even in terms of actual light available, the Raritan estuary was able to convert only about 1% to

TABLE 13. *Summary of various efficiency relationships in the total water column at the Princess Bay station during summer, 1959*

Experiment	$\pi_{0\to z} I_0^{-1}$ (cal/kcal)	$(\pi I^{-1})_{0\to z}$ (cal/kcal)	$(\pi I^{-1}\gamma^{-1})_{0\to z}$ (cal/kcal/μg γ)	$(\rho\gamma^{-1})_{0\to z}$ (cal/μg)	$(\rho\pi^{-1})_{0\to z}$ (cal/cal)
I	7.58	6.12	0.18	0.14	1.36
II	9.35	10.49	0.23	0.10	0.54
III	5.34	7.05	0.15	0.16	1.60
IV	14.58	17.14	0.17	0.09	0.67
V	7.57	6.79	0.22	0.14	2.05
VI	3.74	2.42	0.12	0.10	1.19
VII	17.82	28.71	0.17	0.14	2.49
VIII	8.04	5.64	0.14	0.22	1.12
IX	7.78	5.61	0.38	0.65	1.58
X	13.43	8.83	0.27	0.20	1.13
XI	1.66	0.96	0.04	0.06	2.58
Means	8.81	9.07	0.19	0.18	1.48

producer biomass. Peak efficiencies occurred in experiments IV and VII, with the lowest value in experiment XI.

5. $(\pi I^{-1}\gamma^{-1})_{0\to z}$: This ratio measures chlorophyll activity relative to amount of light transmitted through the water column. The range (Table 13) was from 0.04 to 0.38 cal kcal^{-1} μg^{-1} γ, with a mean of 0.19. The table shows highest γ activity in experiments IX and X and next highest in II and V. The lowest value was recorded in XI, tending to substantiate the idea that deteriorating communities were extant at this time.

These data demonstrate rather clearly that the hypothesis of constant γ activity at unit light intensity is not tenable, presumably because of differences in community composition and variability in physiological state. In the Raritan, chlorophyll collected at 1 ft was usually 2–7 times more active photosynthetically than that found at greater depths (Table 14). This attests further to the vigor of surface communities postulated earlier, and suggests that organisms at deeper levels are older and moribund. Thus, it is concluded that biological stratification in excess of hydrographic stratification can become established and persist in a water column when the flora is dominated by motile flagellates.

This maintenance of a vertical gradient requires a steady energy supply. Because of the shallow compensation depths, any loss in vitality would result in sinking and ultimate retrogression. That such a vitality decrease is marked by γ activity reduction is aptly demonstrated. This occurs, presumably, before final metabolic failure, as indicated by general uniformity of respiration at all depths. Toward the end of the summer, populations of motile *Massartia* began to subside in favor of increased dominance by non-motile forms, *Nannochloris* and *Skeletonema*. This change in composition was reflected in loss of higher surface γ activity in experiments X and XI (Table 14).

6. $(\rho\gamma^{-1})_{0\to z}$: As indicated previously, ρ_z and γ_z generally correlated rather well (compare Tables 6 and 9), suggesting a steady relationship between chlorophyll content and quantity of metabolizing plant biomass. Gillbricht (1951) estimated 1 μg

TABLE 14. *Relative γ activity of samples collected at various depths and resuspended at 1 ft. The ratio used was $\pi_z I_z^{-1}\gamma_z^{-1}$*

Experiment	Collected 1 ft	Collected 5 ft	Collected 9 ft
I	2.3	1.0	1.2
II	2.6	1.0	1.2
III	3.0	1.3	1.0
IV	5.8	1.0	1.4
V	4.4	3.0	1.0
VI	3.3	1.3	1.0
VII	2.5	1.0	1.1
VIII	6.6	1.0	1.3
IX	5.0	1.0	2.1
X	1.8	1.0	1.3
XI	1.4	1.0	1.1

of chlorophyll to correspond to 34.8 μg dry organic matter, or 13.6 μg carbon if a 2.5 : 1 ratio between dry matter and carbon is used. Atkins and Parke (1951), however, indicated considerable variation in number of cells of different species required to yield a chlorophyll unit. Even within the genus *Chlamydomonas*, where organic matter per cell is probably similar, *Chlamydomonas* I and II had, respectively, 202×10^6 and 109×10^6 cells equivalent to 1 mg chlorophyll. If a constant relationship between γ and phytoplankton biomass were found, then $B(Q)$ could be estimated readily by a simple conversion factor.

Data for $(\rho\gamma^{-1})_{0\to z}$ are given in Table 13. In general, variability was not great, the range (excluding experiments VIII, IX, and X) being 0.06–0.16, mean 0.12 cal μg^{-1} γ. Correcting this for a modicum of zooplankton catabolism, we can employ an arbitrary value of 0.10 cal μg^{-1} γ as the criterion for a reasonably vigorous phytoplankton community. Values less than this might be considered to indicate loss of vigor while higher values would denote a younger, more robust aggregation. The least value obtained from the declining communities of experiment XI was 0.06. If we use $|-0.04|$ as the maximum limit of positive departure from 0.10 for a phytoplankton community of utmost vitality, then 0.14 cal μg^{-1} γ represents an arbitrary index of maximum phytoplankton vigor. Values in excess of 0.14 may then be considered due to zooplankton respiration. Applying this criterion, a small zooplankton pulse was evident in experiment III, and a major pulse in experiments VIII, IX, and X. A maximum of 0.66 cal $\mu g^{-1}\gamma$ occurred in experiment IX. The associated grazing is undoubtedly instrumental in bringing about demise of the summer phytoplankton crop.

7. $(\rho\pi^{-1})_{0\to z}$: This efficiency (cost) expresses net community production intensively, and reflects the dynamic state of communities, as follows:

$$\rho/\pi = 1 \quad \text{(steady state)}$$
$$\rho/\pi < 1 \quad \text{(growth)}$$
$$\rho/\pi > 1 \quad \text{(regression)}$$

Cost data appear in Table 13. The range observed was from 0.54 to 2.50 cal cal^{-1} (mean 1.48). Thus, the plankton at this sampling station appear to have sustained a net energy loss during the summer of 1959.

DISCUSSION

The validity of the foregoing results is contingent upon the efficacy of the light and dark bottle method, some of the criticisms and potential limitations of which have been outlined. Based on i) subsequent experiments employing an identical technique now in progress in the York River, Virginia, which indicate positive net production for an even deeper water column, and ii) circumstantial indications that the bacterial problem may be nominal (note decline of respiration with depth, Table 8), the Princess Bay station will be regarded here as truly dystrophic during the period of study. This means that the energy losses must be obviated at some other time and/or place if the system is not to fall stagnant.

The experimental site stands in relation to the general circulation as follows. According to Ayers, *et al.* (1949), flushing is accomplished by mixing resulting from reversing tidal currents, by net translations of water masses due to inequalities between flood and ebb, and by fresh-water flow to the sea. Numerous shoals produce eddies which reduce and deflect tidal currents and inhibit flushing. The normal drift may also be altered by winds which move surface waters before them. The main fresh-water source is the Raritan River. Salt water flows into the bay between Sandy Hook and Romer Shoal (Fig. 1). This water is the uniformly mixed sea water plus Hudson River water of Lower Bay. A sluggish eddy at Old Orchard Shoal impedes exchange between Hudson River and Raritan Bay. On flood tide, salt water from Lower Bay moves westward into the Raritan along the Staten Island shore, then thrusts southwesterly in the vicinity of Princess Bay to a point just west of Conaskonk Point. This tongue of water is presumed to accelerate the seaward movement of fresher water along the south shore while damming back

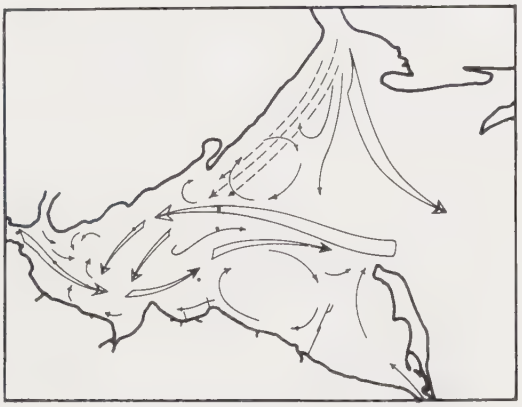

FIG. 3. Schematic diagram of the general circulation of the Raritan–Lower New York Bay system.

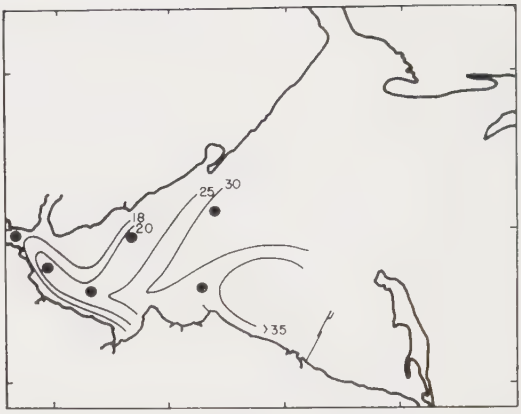

FIG. 4. Mean diversity of net phytoplankton in Raritan Bay between July, 1957 and September, 1958, based on results obtained at the six stations shown. (The diversity index employed was the Shannon (1948) information function

$$H = -N \sum_{i=1}^{m} p_i \log_2 p_i, \text{ where } H \text{ is diversity,}$$

and p_i is the probability for occurrence of the i-th of m species in the aggregation and N is the number of organisms of all kinds per liter. The units are bits $\times 10^{-3}$ L^{-1}.)

low salinity water accumulated at the head of the bay. These general relationships are shown in Figure 3.

It is uncertain how representative the Princess Bay station is of the estuary at large. Mean water depths are comparable. Production rates at the primary level are equally high, as indicated by comparison of the 1957 and 1959 series of experiments. Chlorophyll levels at Princess Bay were not unlike those at other stations during the same season of other years. Consequently, this station probably fairly represents the trophodynamics of mid and upper bay. The following interpretations are based on this assumption.

The Raritan estuary is polluted by both domestic and industrial wastes. Although nutrient levels are high as a result, there occurs a drastic reduction in phytoplankton diversity proceeding upbay from Lower Bay (Patten 1959*b*) following a pattern strikingly similar to that of the general circulation (compare Figures 3 and 4). This suggests an adverse effect of pollution originating, for the most part, at the head of the estuary. During the early part of this study, the Raritan River was so highly polluted that brackish plant species, which normally contribute to diversity and productivity of upper estuaries, were completely non-existent. Consequently, the main contribution to primary production was from the high salinity flora from Lower Bay. The upper estuary is marginal in many respects for these forms, and the indicated dystrophy may be considered to reflect the dying-out of high salinity, pollution-intolerant floras from Lower Bay as they are thrust into the upper reaches of the Raritan.

This does not necessarily imply lack of production at higher trophic levels. To the contrary, Jeffries (1959) has shown the Raritan to sustain a rich and varied planktonic fauna, and an extensive survey of the benthos by Dean (personal communication) also signifies no lack of energy flow to benthic consumers. Thus, there may be postulated a division of labor in the greater New York Bay ecosystem whereby a polluted segment, the Raritan, maintains high biological quality in the pollution-tolerant upper trophic levels, though incapable of any net organic production at the pollution-intolerant primary level. The main energy flux into the Raritan would appear, then, to be not positive net trophogenesis, $(\pi - \rho)$, but rather standing crops, $B(Q)$, from Lower Bay. The lower part of this system may be regarded as having an agrarian

function with respect to the whole; it grows food which is subsequently transported to the upper segment. In return, it may receive from the latter several services which collectively enhance its productive capacity. Adverse pollutants are localized and diluted, while associated nutrients may be transported downbay. Regenerated nutrients may also be transported downbay to help make possible repetition of the cycle.

This differentiation of function at an epiorganismic level suggests for the ecosystem considerable capacity to become organized for optimum biological activity under conditions of environmental adversity. The degree to which the established relationships will be altered by the lifting of pollution from the Raritan River should provide an interesting area for further investigation against the background provided here.

ACKNOWLEDGMENTS

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APPENDIX

The model of information flux in plankton presented earlier (Patten 1961) contained some errors which it is the intent of the following remarks to correct.

In Table 1, flow data were presented which were to represent fluxes in the whole water column (from surface to 15 ft). Instead, the data actually are for *mean* fluxes at any depth. To obtain totals for the whole water column, the figures given for $I_{0\to z}$, $\pi_{0\to z}$, $\rho_{0\to z}$, and $(\pi-\rho)_{0\to z}$ should be multiplied by 4.572, which is the depth in meters.

In equation (14), biomass information in a water column at time t was related to that at time 0 by

$$[B_t(I)]_{0\to z} = [B_0(I)]_{0\to z} \exp \{\phi\, I_0 \delta^{-1}[\tfrac{1}{2}(1+e^{-2\delta z}) - e^{-\delta z}] - \lambda z\} t, \qquad (14)$$

in which the exponent expresses net production from surface to depth z, that is,

$$\int_0^z [\phi\, I_0 e^{-\delta z}(1-e^{-\delta z}) - \lambda]\, dz. \qquad (i)$$

In (i) the left term is gross production and the right term respiration per unit biomass at depth z.

Developing equation (15), it was stated that the exponent of (14) must vanish at the compensation depth, c. This is erroneous. It is net production *at* this depth which vanishes:

$$\phi\, I_0 e^{-\delta c}(1-e^{-\delta c}) - \lambda = 0, \qquad (ii)$$

not, as given in (15), the total net production integrated from surface to c. The depth at which the condition of (15) prevails may be termed the *equivalence depth*, ϵ, and is that depth at which total gross production of the water column above is exactly matched by the total respiration, *i.e.* $\pi_{0\to\epsilon} = \rho_{0\to\epsilon}$. Under the assumption of vertical biological homogeneity upon which the model was constructed, $\epsilon < z$ would signify that the community must experience a net information loss in the water column since $\rho_{\epsilon\to z} > \pi_{\epsilon\to z}$. Similarly, $\epsilon > z$ implies, by definition of ϵ, a net information gain to the community as a whole.

The model from equation (16) on can be made valid by replacing c by ϵ. This invalidates the comparison which was made between model and Raritan Bay data because compensation depths rather than equivalence depths were employed in the computations. Since compensation depths are easily obtained experimentally while equivalence depths are not, it is desirable to express the variables of equations (17–20) in terms of compensation rather than equivalence. This may be accomplished

beginning with (ii) above, from which λ may be expressed as

$$\lambda = \phi\, I_0 e^{-\delta c}(1 - e^{-\delta c}). \qquad \text{(iii)}$$

Substituting into (14), we obtain instead of (16)

$$[B_t(I)]_{0\to z} = [B_0(I)]_{0\to z} \exp = \{\phi\, I_0[\delta^{-1}(\tfrac{1}{2}[1 + e^{-2\delta z}] - e^{-\delta z}) - z e^{-\delta c}(1 - e^{-\delta c})]\}t, \qquad \text{(iv)}$$

where gross production in the water column is

$$\pi_{0\to z} = \phi\, I_0 \delta^{-1}[\tfrac{1}{2}(1 + e^{-2\delta z}) - e^{-\delta z}][B(I)],$$

and respiration is

$$\rho_{0\to z} = \phi\, I_0 z e^{-\delta c}(1 - e^{-\delta c})[B(I)].$$

Since, from (iii),

$$\phi = \lambda\, I_0^{-1} e^{\delta c}(1 - e^{-\delta c})^{-1},$$

and, from (8),

$$B(I) = \rho\lambda^{-1},$$

the expressions for $\pi_{0\to z}$ and $\rho_{0\to z}$ may be written

$$\pi_{0\to z} = \rho\delta^{-1} e^{\delta c}[\tfrac{1}{2}(1 + e^{-2\delta z}) - e^{-\delta z}] \cdot [1 - e^{-\delta c}]^{-1} \qquad \text{(v)}$$

and

$$\rho_{0\to z} = \rho z. \qquad \text{(18)}$$

Net production in the water column becomes

$$(\pi - \rho)_{0\to z} = \rho\{\delta^{-1} e^{\delta c}[\tfrac{1}{2}(1 + e^{-2\delta z}) - e^{-\delta z}] \cdot (1 - e^{-\delta c})^{-1} - z\}, \qquad \text{(vi)}$$

and the cost is

$$(\rho\pi^{-1})_{0\to z} = z\delta e^{-\delta c}(1 - e^{-\delta c}) \cdot [\tfrac{1}{2}(1 + e^{-2\delta z}) - e^{-\delta z}]^{-1}. \qquad \text{(vii)}$$

Expected costs were computed from (vii) and compared with the empirical results from Raritan Bay employing the Kolmogorov–Smirnov test as before. The observed values were found to differ significantly from those predicted by the corrected model.

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GAS EXCHANGE IN A GEORGIA SALT MARSH[1]

John M. Teal[2] and John Kanwisher

ABSTRACT

The oxygen consumption of the undisturbed marsh surface, of well-stirred mud samples, and of above- and below-ground portions of *Spartina* was measured as were oxygen and redox profiles and sediment particle size. All but the uppermost fraction of the sediment was oxygen free and the intensity of reduction was high in all but the best drained areas and was not correlated with amount of reduced material present. The latter was, however, roughly correlated with primary productivity. The rate of energy degradation of the bacteria living in the marsh mud was increased about 25 times when they were supplied with oxygen. *Spartina*, the major marsh producer, consumes more oxygen than any other group. Next in importance are the bacteria of the mud.

INTRODUCTION

There is a broad band of salt marshes running along the southeast coast of the United States which, because of their relative simplicity, make good subjects for ecological research. Most of the primary production can be attributed to *Spartina alterniflora*, the only important higher plant growing on the marshes. The remainder is due to the algae growing on the surface of the marsh mud. Considerable detritus is formed from products of primary production and much of this accumulates on the mud surface producing a black mud, rich in organic matter. In and on this mud live most of the marsh consumers from fiddler crabs to nematodes and bacteria. In this paper we are concerned with the role of the mud and its micro-fauna in the marsh energy budget and also with some of the properties of the mud which are important to organisms living in it.

METHODS

A series of random stations was chosen from a grid drawn on an aerial photograph of the marsh (Fig. 1). Cores were taken at these stations by pushing lucite tubing down into the mud and lifting the tube after stoppering the top. With the bottom then stoppered, the cores were easily brought into the laboratory for oxygen, redox, and gas exchange measurements.

The relative altitude of the stations was determined by measuring the maximum depth of water over the mud at one high tide. Particle size analyses of sediments from 8 stations were done by the pipette method described in the Field Manual of the Allan Hancock Foundation (1958). An estimate of organic content was made by igniting samples in a muffle furnace and correcting for the water of crystallization of the clays, on the assumption that the entire weight loss of the clay sample with the least loss on ignition was due to water loss.

Oxygen profiles down into the mud were made with a polarographic electrode (Kanwisher 1959), pushed into the mud and then moved laterally to determine the oxygen tension at a given depth. This technique is subject to errors of as much as 20% when tested with known tensions, but provides useful estimates where no other method is available.

Below the point where the oxygen electrode indicated anaerobicity the varying degrees of reduction were determined by the redox potential. Hayes, Reid, and Cameron (1958) have shown that the measurement of redox potential of sediments is complicated by the presence of H_2S which acts on the Pt electrode in an irreversible manner. Large electrodes gave better results than small ones. They also reported that different types of sediments produce curves of redox potential against depth that are not comparable. Our study dealt with

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FIG. 1. Aerial photograph of Georgia salt marsh showing sites used in this study. Streamside marsh is narrow dark band along creeks, levees show as broad irregular grey just beyond. Distance from site 16 to site 17 is 0.4 km.

sediments that were all fairly similar or at most represented samples along a gradient and we used a relatively large 7-mm diameter electrode. Although the actual values we obtained may not accurately indicate the true state of reduction in the samples, they are a useful relative measure. We may note that in general the muds were brown in color at potentials above +0.100 v; grey from +0.100 v to –0.100 v; and black at potentials below –0.100 v. Also in the black regions the presence of H_2S was indicated by its odor. Duplication was poor in the less reduced cores that were visibly nonhomogeneous. Values from the black reduced muds could be repeated within 0.02 to 0.04 v.

The oxidation–reduction profiles were obtained by pushing the 7-mm Pt electrode slowly into the cores. Because of drift sev-

eral minutes were allowed at each depth before each reading. A calomel electrode at the edge of the core completed the circuit. The pH of the muds varied from 6.5 in black areas to 7.4 in brown. This whole range would affect *r*H, when computed from *Eh*, by no more than 40–50 mv.

Such redox potentials are related to the intensity of reduction, but tell nothing about the amount of reduced material present, *i.e.* the amount of oxygen debt of the mud. Two methods were developed for such determinations. A 1-cm^3 sample of mud was taken with a small coring tube with a minimum of exposure to air. It was extruded into a 25-ml container of sea water. An oxygen electrode was immediately inserted for a stopper and the disappearance of the dissolved oxygen was recorded while the contents were vigorously stirred magnetically. In this way the mud particles were quickly dispersed throughout the water and given maximum access to oxygen. When the dissolved oxygen had nearly disappeared from the bottle, the stopper was removed, pure oxygen bubbled gently through the water for a few seconds to renew the supply, the stopper replaced and recording continued. Formalin was sometimes injected midway in a run to stop any oxygen consumption by living organisms and provide a way to compare chemical with biological consumption. Formalin is a reducing agent and so might increase oxygen consumption. Controls indicated no such effect.

In addition 1-cm^3 mud samples were placed in 20-ml syringes with 1 ml of sea water and a magnet. When the magnet was spun with a stirrer the mud slurry was thrown about in the syringe and aerated. Gas samples withdrawn at intervals were analyzed with an accuracy of 0.02% for O_2 and 0.015% for CO_2 (Scholander 1947).

Oxygen and CO_2 gas exchange at the mud surface was determined on 4-cm and 30-cm diameter cores brought into the laboratory. These were capped with several centimeters of air above the mud and serial gas samples were withdrawn with a syringe. Oxygen consumption and CO_2 production were determined with the ½-cc analyzer. As a check on this procedure several cores were fitted onto Scholander type volumetric respirometers and oxygen uptake was followed (Scholander, *et al.* 1952). RQ's were calculated from runs where both respiratory gases were measured.

Since the gas exchange of the marsh mud takes place under water for a considerable part of the time some measurements were made with cores in which the mud surface was covered with water. A layer of oil floated over the water reduced exchange with the air to a negligible amount. Water samples were taken at intervals and oxygen determined with the gasometric technique (Scholander, *et al.* 1955). If the water was not stirred an oxygen gradient developed. The layer just over the mud surface could become very depleted, or even anaerobic, in as little as one hour. This was prevented by stirring gently with a wire loop inserted through the oil layer. Oxygen uptake under these conditions was not significantly different from that measured in air and will not be referred to again. Very few places on the marsh had water stagnant enough to develop oxygen gradients at the mud surface.

The cores were taken so as to exclude crab burrows, mussels, snails, or *Spartina* plants. Data for gas exchange of the entire marsh community was obtained by following changes in composition of air under a gas-tight 5,000-cm^2 plywood box pushed into the mud to seal the edges. A rubber stopper in the top allowed samples to be withdrawn by a syringe. A cover of aluminum foil minimized heating by the sun. To obtain a measurable change in a few hours the air space under the box was kept to 15–20 cm. The plants were thus bent over.

The respiratory contribution of the above-ground parts of *Spartina* plants was determined by repeating runs after cutting the plants off at the mud surface. Independent checks were made using the ½-cc analyzer to follow the gas exchange of sections of *Spartina* leaf and stem enclosed in a 100-ml syringe.

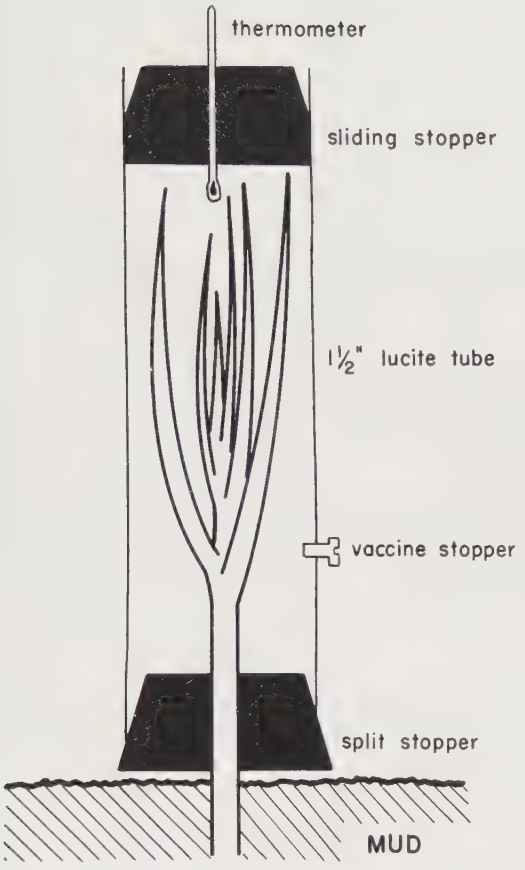

FIG. 2. Diagram of lucite tube used for comparing respiration of above- and below-ground parts of *Spartina* plant showing plant before it was cut off above lower stopper.

The *in situ* box measurements with the *Spartina* tops removed showed an oxygen consumption per unit area above that of the core samples even when allowance was made for the crabs, mussels, *etc.* Since the mud was anaerobic it seemed logical to suppose that the root system of the *Spartina* might obtain its oxygen through the aerial parts of the plant. Such root respiration was determined by measuring the gas passing into the cut-off stems. A plant was sealed in a section of lucite tube with a stopper at the top and another at the bottom bored to fit the stem snugly (Fig. 2). Respiration was measured with the plant intact and after the stem had been cut off just above the bottom stopper. The respiration of the underground parts of the plants could then be expressed as a percentage of the respiration of the intact plant.

RESULTS

The marsh profile with sampling sites is shown in Figure 3. The horizontal scale is distorted non-uniformly. The left side of the figure represents a tidal creek bank where the mud near low-water level contained a large proportion of sand and where *Spartina* did not grow. *Spartina* began to occur at about 1.2 m above mean low water where it grew to about 3 m in height, with the plants close together forming a dense luxuriant "streamside marsh." The break in elevation represents the little cliffs frequently found as a result of slumping of the marsh due to stream undercutting of the banks. The next series of stations were representative of the areas from the streamside marsh up to the top of the natural levee which forms along all of the larger tidal rivers and creeks. The *Spartina* grew to a height of about 1 m. In the levees, as on the rest of the marsh where the typical *Spartina* growth occurs, there was very little sand and the mud consisted of about equal parts silt and clay, most of the silt occurring in the 16–8, 8–4, and 4–2 μ fractions and 80% of the clay particles in the <1 μ fraction. After spring tides mud from all sites was about 65% water (expressed as a fraction of wet weight).

Behind the levees there is usually an extensive area where the *Spartina* is short, 0.5–0.7 m. Station 2 represented such a site at the beginning of a tidal creek well above low-water level. The two stations on the right represented places where the marsh abutted on the land. Here the soils became much more sandy, the *Spartina* was stunted, and shared the ground with other plants (*Distichlis, Salicornia, Sporobulus*).

Except for areas near low water, the fineness of the sediments prevented extensive movements of the interstitial water in spite of the changing hydraulic pressure due to the tides. A depression in the mud drains very slowly at low tide. In fact the entire surface of much of the short-*Spartina*

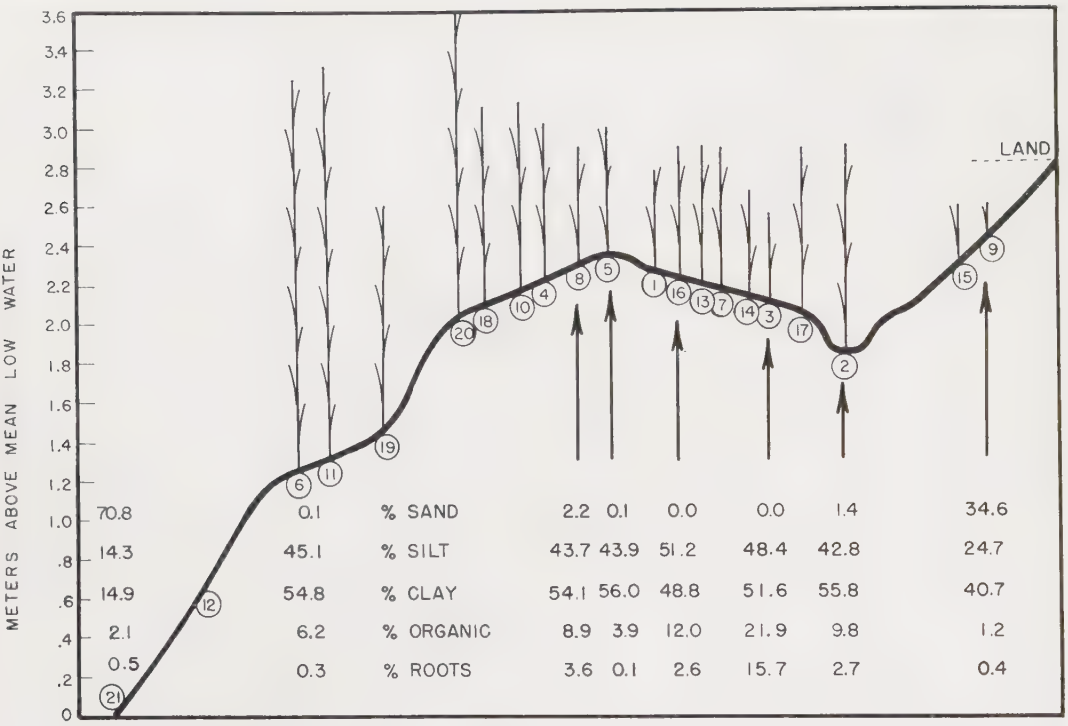

FIG. 3. Diagrammatic salt marsh section indicating elevation relative to low tide and height of mature *Spartina* plants at sampling sites. Sediment cores of 5 cm diameter from the upper 10 cm were used for particle size analysis. Horizontal scale is distorted non-uniformly.

marshes, behind the levees, had water on the surface continually during the spring tide period when drainage was so slow that it was not complete before a subsequent high tide arrived. Conversely some of the levee marsh dried out completely on the surface during neap tide intervals and the mud became very hard and caked.

The organic content of the muds was lowest near the low-water level and near the land, and was highest in the short *Spartina* marshes. The amount of roots, *etc.* in the mud was appreciable only in the higher parts of the marsh. In the short-*Spartina* areas this formed a peat-like layer from the surface to about 30 cm deep in which the mud particles were tightly held together by the roots. Aside from the roots, most of the organic matter was found in the silt fraction.

The Intensity of Reduction

We have divided the stations into three groups: Figure 4a includes sites on the outer sides of the levees; 4b, sites from the short-*Spartina* marshes; 4c, sites from the creeks and creek banks. The levee stations (Fig. 4a) except for No. 10 were relatively well drained with a firm mud. No. 10 was on the levee of a small creek where the mud was quite soft and resembled the creek banks more than the other levees. In spite of the relatively high redox values the oxygen electrode indicated a measurable oxygen tension down to 2 cm only in core 9. At other stations in the marsh there appeared to be no oxygen at a depth of more than a few millimeters. A similar situation was found in lakes by Hayes, *et al.* (1958). They thought mixing of the bottom was involved in the formation of the surface-"oxidized" but oxygen-free layer.

The creek stations, with the exception of No. 11, all had the very soft mud typical of these areas and showed a sharp decrease in potential just below the surface. These gave cores with the blackest mud and the strongest H_2S smell. No. 11 was unusual in

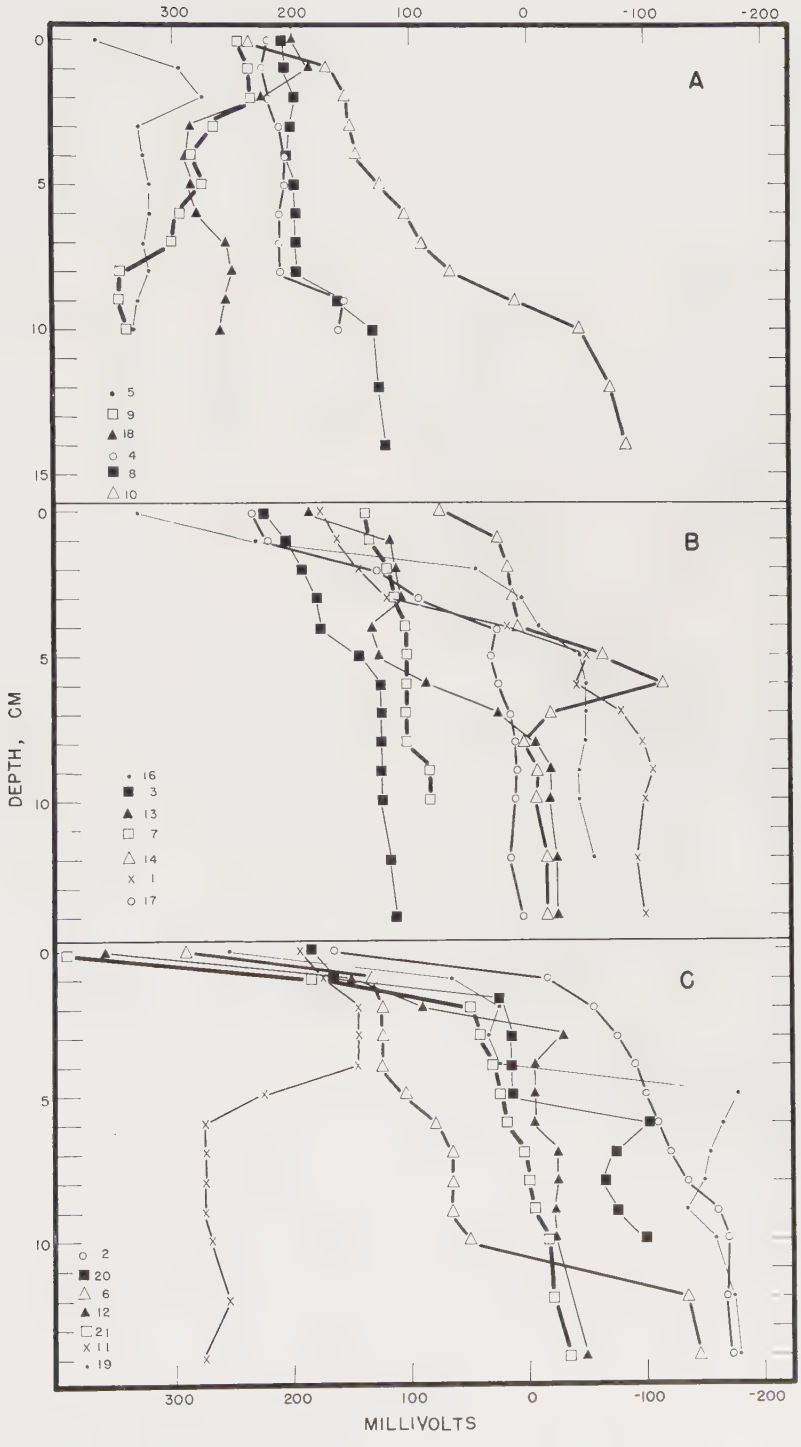

FIG. 4. Redox profiles from salt marsh sampling sites divided into three groups: a—sites on outer sides of levees; b—sites from short-*Spartina* marshes; c—sites from creeks and creek banks.

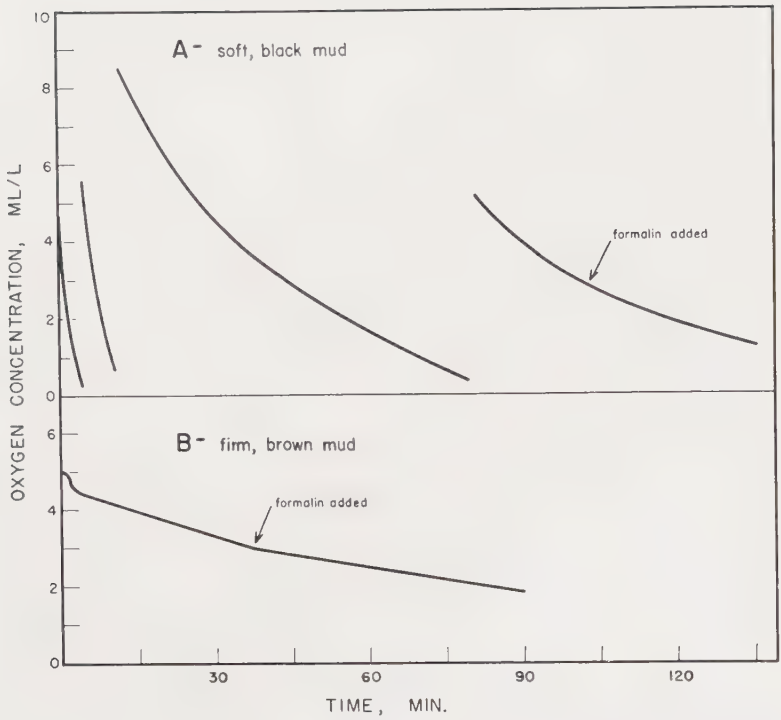

FIG. 5. Typical curves of oxygen consumption of well-stirred mud obtained with the oxygen electrode. Sample A is from a creek bank, B from a levee.

that it had a firm substratum and a steep bank next to the coring station where the stream was undercutting the bank.

The Pt electrode consistently indicated 2–3 cm lumps of black mud distributed in the brown mud layers by a potential of –0.100 v or below. This gave us confidence in the relative shapes of the curves indicated by such electrode potentials. Examination showed that these lumps had a piece of partly decayed plant material in their center. It seems reasonable that bacteria were living in the organic material, oxidizing it and consequently reducing the surrounding mud.

The redox profiles for the sites in the short-*Spartina* marshes (Fig. 4b) are intermediate between those for the levees and creek banks. Some show a definite break in the curve near the surface and others do not. This difference was correlated with the presence of a root mat and the hardness or softness of the mud. With a well-developed mat and firm soil, the burrows and holes in the soil could remain open, while in the soft areas openings in the mud collapsed and filled in as the tide rose.

It seems obvious from Figure 4 that small differences in redox potential in natural muds are not significant. But a series of determinations can be useful for the comparison of sediments especially as regards the change from oxidized to reduced conditions (Pearsall and Mortimer 1939), and when combined with other measurements, particularly in areas like salt marshes where the sediments are visible. Variations in such a situation may have meanings that would be puzzling to anyone forced to sample the mud at random from a position where he could not see it, as for example when sampling underwater sediments from a boat or ship.

The Quantity of Reduced Material

Figures 5a and b show two typical curves of mud oxygen consumption obtained with the oxygen electrode. The first

TABLE 1. *Oxygen uptake in well-stirred mud samples. "Final rate" is taken from the curve after sufficient time has elapsed for the rate to become relatively constant. Formalin decreases refer to final rates and not initial rates. Curves illustrated in Figure 3 are indicated*

Core	Depth (cm)	Initial rate—1st 15 min (mm^3/hr/ml)	Final rate (mm^3/hr/ml)	Decrease after adding formalin
6	0–1	260	–	–
	0–1	310	65	35%
	1½–2½	340	90	22%
	1½–2½	320	70	–
	3–4	230	80	0
	3–4	250	105	0
	4–5 (Fig. 3a)	680	70	0
	12–13	1,070	150	–
21	0–1	110	80	21%
	2½–3½	650	–	–
	4–5	350	90	31%
2	0–1	510		
	2½–3½	620		
	4½–5½	460		
4	0–1	220	85	27%
	3–4	730	75	51%
	6–7	470	105	21%
8	0–1	60	80	21%
	2–3	90		
	4–5 (Fig. 3b)	100	90	31%
7	0–1	131	122	50%
	1½–2½	336		
16	0–1	188	61	25%
	0–1	114	73	33%
	4–5	291	61	33%

is a sample from a creek bank station with very soft, black mud. Oxygen uptake was very rapid at first and gradually declined with time. There was no appreciable change in rate when formalin was injected. Figure 5b was derived from the 4–5 cm level of site 8 which represents the extreme case of a well-oxidized sample. It showed a low rate of oxygen uptake and in which living organisms or at least systems susceptible to formalin poisoning accounted for approximately ⅛ of the rate. A summary of the results is given in Table 1. The uptake varied in the first 15 min from 60 up to 1,000 mm^3 O_2/hr/ml mud; the highest values were obtained from deep levels of the soft, black cores; the lowest from the upper layers of the better oxidized sites.

The rapid initial oxygen uptake in these well-stirred mud samples is a convenient indicator of the comparative amount of reduced material present, at least in the salt marsh. Since all marsh sites should have the same sorts of reduced compounds, uptake by the easily oxidized portion will be proportional to total chemical uptake or oxygen debt.

Attempts to poison the initial rapid uptake with formalin were unsuccessful. Apparently the oxygen consumption during this phase was predominantly chemical. This conclusion is supported by the experiments done in the syringes. These showed that CO_2 production was initially very low in relation to the oxygen consumed. This would be the case with oxidation of metals, H_2S, *etc.*

The final or slow phase presents a contrast. There was usually about a 30% reduction in oxygen uptake following addition of formalin and the RQ measured in syringes was normal for respiratory processes, between 0.7 and 1. Occasionally in deeper levels of highly reduced cores there seemed to be no biological activity even in the slow phase.

Undoubtedly the biological part of the oxygen consumption was present from the beginning of the runs, but was overshadowed at the start by the high chemical demand. As the more highly reduced materials were oxidized and the chemical demand slowed, the biological respiration became relatively more important. Presumably if the experiments had been continued long enough only the biological part would have remained. This proved to be the case in similar experiments done with Nova Scotia lake sediments.

Gas Exchange of the Mud Surface

The results of 37 determinations of gas exchange across the mud surface of cores are summarized in Table 2. The cores are listed approximately in the order: creek bank, streamside marsh, levee, short-*Spartina* marsh, landward marsh edge. There

TABLE 2. *Oxygen consumption and RQ's of cores with undisturbed surfaces from Georgia salt marsh*

Site	O_2 consumption ($mm^3/cm^2/hr$)	RQ
21	3.6	–
2	5.0	.72
6	6.8	.54
11	5.6	–
19	5.5	–
18	5.1	–
20	4.3	–
10	5.3	.78
4	4.0	–
8	5.0	.70
5	7.4	.50
1	3.4	.96
13	4.4	–
16	4.4	–
7	5.5	–
14	2.9	–
3	8.4	.79
17	3.5	–
15	6.0	–
9	7.0	–

was no discernible trend within the values and all lay fairly close to the mean 5.2 ± 1.8 mm^3 $O_2/cm^2/hr$. This value represents respiration of bacteria, nematodes, *etc.* in the surface layers of the mud plus whatever chemical oxidation took place on the surface.

The 5,000-cm^2 box was used at three sites in the marsh (Table 3). Column 4 shows that cutting the grass off at the mud surface still resulted in an oxygen uptake per unit area much higher than that found with small cores. The underground parts of the plants showed oxygen uptake rates of 82, 73, 70, 67% of the above-ground portion. We have subtracted an amount equal to 73% of the grass respiration to give the results in the final column of Table 3. This represents the respiration of the mud and contained animals, macro-fauna such as crabs, mussels, and snails, and also the respiration of bacterial populations of such dead *Spartina* leaves as were lying on the surface of the mud.

Since the field data from the box in some cases were collected over several days while the temperature was fluctuating, it was possible to calculate a Q_{10} of 1.7 for the mud with the animals and grass roots.

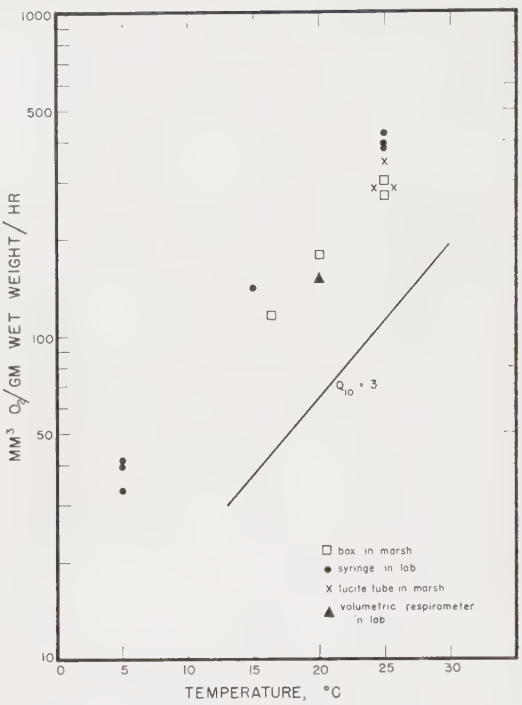

FIG. 6. Respiration of *Spartina alterniflora* from Georgia salt marsh plotted against temperature. Line indicates slope at which $Q_{10} = 3$.

RQ's were calculated for the contents of the boxes giving a figure of 0.68 with the grass present and 0.82 with it removed.

Respiration of Spartina

Respiration determinations of undisturbed *Spartina* plants in the marsh were combined with laboratory measurements on parts of plants to give Figure 6. The data from the three types of measurements do not show significant differences and indicate a high Q_{10} of about 3.

DISCUSSION

The mud of the Georgia salt marshes is, over most of the marsh area, a very finely divided substrate holding from 50–70% of its weight in water when saturated. As has been noted, this water moves through the mud only slowly and the movement that does occur can be assumed to take place mostly through crab burrows, worm holes, and root holes. The bulk of the mud must receive oxygen, if at all, by diffusion from

TABLE 3. *Salt marsh respiration under ½-m² box. Values are given as mm³ O_2 used/cm²/hr*

1 Site	2 Temp.	3 Intact surface	4 Without *Spartina*	5 *Spartina* rate col. 3 – col. 4	6 Root rate	7 Mud rate col. 4 – col. 6
Creek head 1-m *Spartina*	17°	20	13	7	5.1	7.9
Short-*Spartina* marsh	25°	32.8	18.5	14.3	10.4	8.1
Levee marsh	20°	31.5	18.6	12.9	9.4	9.2

the surface. That this is a very slow process is evidenced by the absence of oxygen, presence of H_2S, and highly reduced conditions found below the top centimeter in all but the highest, best drained stations.

All of the primary production of organic matter occurs on or above the surface of the marsh and comes to lie on the surface as the plants die.

Surface animals can use the oxygen of the air and water to metabolize this potential food. But as soon as the organic material has been buried more than a few millimeters by either animal movement or further deposition on top, it is in an anaerobic environment. The bacteria and nematodes living there can only use it as food by parallel oxidizing and reducing reactions. The result is an accumulation of reduced end products such as CH_4, H_2S, and ferrous compounds.

The increasing degree of reduction (lower redox potential) suppresses biological activity by reducing the number of tolerant species and by limiting the number of reactions that can occur. The reduced materials will be transported slowly upwards by diffusion in a similar manner to the way in which oxygen moves down. They may form a food source at some higher level (lower redox potential) or they may combine with oxygen directly. We can assume the salt marsh soil to be relatively stable; that is to say, no great disturbances occur often enough to be important in terms of the life cycles of the organisms or the rates of the chemical reactions involved. This produces a horizontally stratified system which is highly reduced deep in the mud and has abundant free oxygen available on the surface. Between these two extremes diffusion and mixing will occur. If accumulation of organic matter is also relatively slow, oxygen will be consumed at a rate proportional to degradation of free energy by the organisms whether they are living at the surface or down in the mud. A kind of countercurrent exchange will prevent many of the reduced substances from escaping to the atmosphere. For example, H_2S is rarely smelled on the surface although it is present in large amounts 1 or 2 cm down; RQ's close to one also substantiate this. If reduction over the entire mud column and oxidation near the surface were not balanced, one would expect to find RQ's considerably greater than one reflecting the anaerobic production of CO_2. We can reasonably conclude then that the rate of oxygen consumption per unit area at the surface is an integrated measure of the energy degraded in the mud column. There remains a possibility that chemically resistant reduced compounds such as CH_4 diffuse all the way to the surface and are lost to the atmosphere.

If there is no appreciable accumulation of reduced material per unit time in the salt marsh soils, the actual amount of reduced substance present will have no relation to the biological activity, but will be related only to the ability of oxygen to penetrate into the mud. With an appreciable accumulation the figures for oxygen uptake per unit area of surface will have to be corrected to give a true measure of degradation in the mud column. The uptake of stirred mud from deep in the core divided by the time required for its accumulation would provide the necessary correction.

We might expect a positive relation between primary production or rate of dep-

osition of the products of primary production and the amount of reduced material in areas with comparable oxygen supply. Figure 3 and Table 1 show some correlation between height of *Spartina* growth and the initial uptake of oxygen in stirred cores. Under non-tidal conditions, both in fresh and salt waters, where the supply of oxygen to the sediments is similar and where deposition and accumulation of reduced substances is appreciable, the measure of oxygen debt would serve as a useful index of biological activity in the mud.

Kato (1956) also measured the oxygen uptake by marine muds which were stirred and then allowed to settle. He similarly found that the chemical demand representing former biological activity exceeded the demand by living organisms.

Mortimer (1942) showed that a relation between the degree of reduction and amount of reduced material implies a correlation between redox potential and the square root of capacity to consume oxygen. We found no significant correlation using the values of initial consumption in stirred cores from Table 1 and the redox values in Figure 4.

There was no recognizable trend with depth or station in the data for respiration sensitive to formalin poisoning in the mixed mud samples. The average value was 28 $mm^3/hr/cm^3$ with a standard deviation of 16. The value for respiration of undisturbed mud cores was 5.2 $mm^3/hr/cm^2$, of which 0.2 $mm^3/hr/cm^2$ represents the respiration of the nematodes and annelids (authors' unpublished data) leaving some 5.0 $mm^3/hr/cm^2$ which might be attributed to the bacteria. If we assume that the average for the stirred cores holds to a depth of 5 cm (see Table 1), the oxygen uptake of the stirred mud would be 25 times that of the undisturbed core. Since some of the formalin-sensitive oxygen uptake may have been due to extracellular enzymes preserved by the normal reducing conditions, we can conclude that the bacteria exposed to oxygen consumed energy somewhat less than 25 times as fast as when in anaerobic conditions. It is probable that bacteria slow their metabolism considerably to compensate for the reduced free energy available under anaerobic conditions. They commonly reduce their metabolism under harsh conditions, *e.g.*, with spore formation. We know little about possible similar behavior of animals such as the nematodes which also exist anaerobically in the mud.

As we have pointed out above, the respiration of the undisturbed mud, excluding animals, was 5.0 mm^3 $O_2/hr/cm^2$. This value multiplied by the marsh area would not give the oxygen consumption for the marsh mud as a whole, because there are other surfaces through which oxygen is exchanged besides the visible mud surface, principally the burrows of crabs. The data from the boxes placed on the marsh included this exchange and gave an average of 8.4 mm^3 $O_2/hr/cm^2$ (Table 3). When we subtract the respiration due to the populations of animals such as crabs, nematodes, snails, *etc.* (Teal 1958 and authors' unpublished data) the remainder of 6.4 $mm^3/hr/cm^2$ represents the true areal value for the marsh. One can calculate that animal burrows made up 22% of the surface area of the mud if gas exchange was proportional to surface.

If we compare the value of 5 mm^3 $O_2/cm^2/hr$ for the bacteria with some of the values obtained for other marsh organisms, we find that aside from the *Spartina* which uses more free energy than bacteria, all the other organisms use less. The fiddler crabs which are the most conspicuous and abundant larger animals use on the average only 0.32 mm^3 $O_2/cm^2/hr$ and all of the herbivorous insects, crabs, mussels, snails, nematodes, and annelids together use 1.6 mm^3 $O_2/cm^2/hr$ on the average throughout the year (Teal 1958). In the marsh economy only the *Spartina* is more active (Table 3), using more than 3 times as much oxygen as the mud. In summer when the marsh grass crop is larger and the temperature higher the difference might well be greater. The producers are the most important consumers in the marsh. The bacteria are second and they account for more of the energy than all the remaining organisms together.

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PHOSPHORUS BUDGET OF A MUSSEL POPULATION[1]

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ABSTRACT

The phosphorus budget of a *Modiolus demissus* population in a Georgia intertidal salt marsh was studied. Percentage phosphorus in mussel bodies decreased from 1% of the dry weight in small individuals to 0.6% in adults. There was also a seasonal variation in phosphorus content of adults; the maximum occurred during spawning early in September, the minimum in November. The standing crop of phosphorus in the population was 37.2 mg P/m^2, the body fraction comprising 67%, the shell 30%, and the liquor 3%. Prorated losses and elimination rates (μg P/m^2 day) of the population were: mortality 21; gametes 11; dissolved organic 23; phosphate 260; and feces 460. Quantities of phosphorus present in natural marsh water (mg P/m^2) were: particulate 14; phosphate 19; and dissolved organic 6. The mussel population removed 5.4 mg P/m^2 of particulate phosphorus and 0.07 mg P/m^2 of phosphate daily, of which 0.78 mg P/m^2 was required as food and 4.7 mg P/m^2 was deposited as pseudofeces. The turnover time of phosphorus in the population was 115 days. The major effect of the population on the ecosystem was the removal of particulate matter from sea water; the turnover time of the particulate phosphorus in the water was 2.6 days under the supposition that the mussel population was the only agent involved. Mussels are more important as biogeochemical agents than as energy consumers.

Determination of the participatory turnover time of a substance via any route in a system sets the maximum turnover time of that system. Determination of the turnover time of a substance for an entire system fixes the maximum flux rate via any one route in the system.

INTRODUCTION

Phosphorus is indispensable in the biosphere because it is involved in fundamental metabolic processes of energy transfer, both respiration and photosynthesis (McElroy and Glass 1951, 1952). There is available to the organisms which make up the biosphere only a limited amount of the phosphorus which they need for their life processes. Phosphorus passes from the environment to the biotic community during plant growth, from organism to organism by grazing, predation, and parasitism, and eventually back to the environment by excretion or death and decomposition. There are also many strictly chemical transformations and physical or geological movements of phosphorus and its compounds. Hutchinson (1952) has reviewed the biogeochemistry of phosphorus, its occurrence, abundance, and pathways in the lithosphere, atmosphere, and hydrosphere. The present study was undertaken to determine the rate at which phosphorus flows through a population of the mussel, *Modiolus demissus* Dillwyn, in an estuarine, intertidal salt marsh near Sapelo Island, Georgia, and to evaluate the effect on the ecosystem of this particular pathway.

Kuenzler (1961) discussed the population density and distribution, the recruitment, growth, and mortality rates, and the energy flow of these mussels. *Modiolus* is a typical filter-feeding lamellibranch, ingesting some of the particulate matter from sea water, but rejecting a large part as pseudofeces. Recent workers disagree concerning the ability of animals to obtain nutritionally important quantities of dissolved organic matter from sea water (Jørgensen 1955; Collier 1959; Stevens 1960). Possible large differences in the extraction capability of different species and the differing concentrations of dissolved organic matter in different localities make generalization hazardous. The ability of lamellibranchs to obtain phosphate, however, directly from sea water was demonstrated by Ronkin (1950) and Pomeroy and Haskin (1954).

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Hutchinson (1957) gave a detailed survey of work on the phosphorus cycle in fresh-water lakes. An understanding of cycles in fresh-water communities is beginning to be reached, largely through employment of radio-phosphorus as a tracer (Hutchinson and Bowen 1947, 1950; Coffin, *et al.* 1949; Hayes, *et al.* 1952; Hayes and Phillips 1958). The phosphorus cycle in the sea has not been investigated nearly as thoroughly. Barnes (1957) reviewed current knowledge of the cycles and regional distribution of phosphorus in the sea, and Emery and Stevenson (1957) reviewed studies of phosphorus in estuaries and lagoons. Bruce and Hood (1959) used P^{32} and a plastic bag to study *in situ* phosphorus exchanges between water, particulate matter, and mud of a Texas bay. Pomeroy (1960) reported the concentrations, turnover rates, and residence times of phosphate in estuarine, coastal, and offshore marine waters of the Georgia coast. The general distribution of the element and the outlines of its circulation in the sea are now known, but apparently no one has investigated intensively the phosphorus budget of any marine population.

I wish to thank Dr. E. P. Odum and Dr. L. R. Pomeroy for their many helpful criticisms during the progress of this work. I am grateful to Dr. R. J. Conover for also reading and criticizing the manuscript.

METHODS

Phosphorus available to Modiolus

The total quantity of phosphorus available to the mussel population in its food is primarily a function of the size-range of food particles filterable and ingestible, the concentration of these particles in the sea water flooding the marsh, and the volume of sea water per unit area of marsh.

The size range of ingestible particulate matter was determined by allowing mussels acclimated to the laboratory to feed upon suspensions of graded particles of dried *Spartina alterniflora* leaves for 20 to 30 min. The mussels were then opened and the stomach contents were drawn out for microscopic examination. The effectiveness of filtration for each range of particle size was thus estimated.

The amount of particulate food available to the mussel population in marsh water was estimated by a filtration method similar to that described by Goldberg, *et al.* (1952). Known volumes of natural water from the marsh and tidal creeks were collected during flood tide. These samples were passed through a 164 μ screen (to eliminate particles previously shown to be too large for food) and then through a tared cellulosic membrane filter (Millipore) of 0.45 μ pore size. Then the membrane filter was washed with distilled water, dried, and reweighed, giving the total weight of particulate matter in the 0.45 μ to 164 μ size range. The concentration in the water multiplied by the volume of water covering the marsh at high tides equals the maximum amount of particulate matter available to the mussels.

The phosphorus present in natural marsh water was determined as three different fractions: 1) the particulate; 2) the dissolved organic; and 3) the dissolved inorganic, or phosphate. The two dissolved fractions were determined on membrane-filtered water samples. All samples were prepared in duplicate and analyzed by the methods of Hansen and Robinson (1953).

The volume of sea water from which mussels obtain their food depends upon the depth of the water, and this depends upon the elevation of the marsh. Kuenzler (1961) showed the relationship between marsh elevation and water depth, and the area covered by different marsh types.

Diatom cultures

The diatoms needed for feeding experiments were grown in mass culture. A unialgal culture of a *Nitzschia*-like diatom (size 4×40 μ; hereafter designated "Nitzschia") was used for most of the feeding experiments, but a mixture of two diatom species (sizes $5 \times 10\mu$ and $10 \times 27\mu$) was also used. Bacteria were sometimes present in the culture, but their mass was probably less than 5% of that of the diatoms. Centrifugation of the algae from the culture medium prior to feeding the mussels probably further re-

duced the number of bacteria since they would not be thrown out of suspension as effectively as the diatoms. Labeled diatoms were prepared by adding P^{32} to the cultures several days before they were fed to the mussels. Some bacteria are rich in phosphorus (Buchanan and Fulmer 1928), and some are also able to take up phosphate from sea water (Renn 1937). Bacteria in the diatom cultures were almost certainly labeled with P^{32}, but their effect on the experimental feeding of *Modiolus* was probably small.

Phosphorus in mussels

Phosphorus in mussel tissue was determined by the method outlined by Lindner (1944). This consisted of oxidation of the material in sulfuric acid and hydrogen peroxide, followed by a colorimetric determination of phosphorus based on the formation of the blue phospho-molybdate ion. Light absorbency was measured by a Beckman DU spectrophotometer at 650 mμ, 10 min after addition of the last reagent, and compared to a standard curve. Samples of shell required the addition of hydrochloric acid after oxidation in order to dissolve the calcium salts. This HCl was neutralized before bringing the solution to its final volume.

Feeding rates

The rate at which *Modiolus* feeds was estimated from the rate of decrease in radioactivity as the mussel filtered a suspension of P^{32}-labeled diatoms. The general method was described by Chipman and Hopkins (1954). The mussel was placed in 3 L of sea water in a battery jar for several hours so that it might become acclimated and filter this water free of suspended matter. The water temperature was maintained at 10°C in February and at 25°–30°C in August experiments. The labeled diatoms were centrifuged from the culture medium, washed with a small amount of sea water to eliminate the dissolved P^{32}, then resuspended in sea water. The accumulated feces and pseudofeces were carefully siphoned from around the mussel, and the suspension of diatoms was added. The aerator oxygenating the water for the mussel also ensured homogeneity of the suspension and helped prevent settling of the diatoms. Battery jars without mussels were used to determine the diatom settling correction. One-milliliter samples were taken immediately after addition of the diatoms and at 10-min intervals thereafter. The samples were dried on planchets and their radioactivity measured by means of an end-window G-M tube connected to a scaler. Since the P^{32} was almost completely confined to the diatoms and bacteria, decrease in activity corresponded to decrease in number of cells in suspension.

The quantity of water effectively cleared was calculated by a formula similar to that used by Chipman and Hopkins (1954):

$$r = \frac{(\ln C_0 - \ln C_t)(V)}{t}$$

where r is the rate (L/hr) at which the suspension is swept clear of particles; V is the volume of suspension in liters; and ln C_0 and ln C_t are the natural logarithms of the radioactivities of the samples (cpm/ml) at the beginning and after 5 hr, respectively. Since the decrease in activity closely approached linearity on a logarithmic scale during the first 30 min, the filtration rate was determined by using C_0 and C_t calculated from the least squares regression of the natural logarithms of radioactivity against time.

Feces, passed while the animal was in the labeled suspension, were periodically siphoned from the bottom, briefly washed in distilled water, and placed on planchets to determine radioactivity. At the conclusion of the 2- to 4-hr feeding period, the mussel was transferred to ordinary sea water for 10 to 12 hr and then killed for body analysis or discarded. The radioactivities of feces and of dissolved excreta passed during the postfeeding period were measured. The bodies of some mussels were dried, weighed, and wet-ashed in hot H_2SO_4 and 30% H_2O_2. This solution was neutralized and aliquots of it were dried on planchets to determine the quantity of P^{32} assimilated by the mussel. Pieces of the

shell of some mussels were arranged on planchets so that 80% to 90% of the bottom of the planchet was covered, and these activities were measured. Correction was made for loss due to self-absorption in the shell and for coincidence and decay losses when necessary.

To determine the rate at which food passed through the gut, the percentage of the maximum rate of elimination of fecal P^{32} was computed for each 30-min period after feeding. Averages of these percentages for all 10 experiments were plotted against time. The integral of the curve of rate of elimination gave the average length of time that material from one feeding remained in the gut.

The rate of uptake of phosphate by *Modiolus* was determined in the same manner as the filtration rate, except, instead of labeled diatoms, about 28 μc of $P^{32}O_4$ was measured into the sea water after the mussel had filtered out the suspended matter. The first experiment lasted 2 hr, the second lasted 2 days.

Phosphorus elimination rate

In addition to the data gained from the P^{32} experimental feedings, the rate of phosphorus elimination was measured using analytical chemical techniques. The method was patterned after that of Pomeroy and Haskin (1954). Experiments were run at three temperatures, 6°, 15°, and 24°C, during the periods January 9 to 13, March 28 to April 4, and May 7 to 11, 1959. The experimental temperatures approximated environmental temperatures in the marsh on those dates. The mussels were collected from the marsh a few hours before beginning the experiment and were thoroughly scrubbed and rinsed. Measurements were made of the three previously described phosphorus fractions in the supernatant of sea water which had been held without stirring for about a month. Two to five mussels of approximately the same size were placed in each battery jar with 0.5 to 2.0 L of this sea water supernatant. The jars were placed in a temperature-controlled water bath and the sea water in the jars was aerated. The mussels almost always opened and began pumping shortly after being placed in the jars; those experiments begun with mussels which later failed to open were discarded. After 24 hr the inside of each battery jar was vigorously scrubbed with a rubber policeman and the sea water and particulate matter were thoroughly mixed. Finally, the three phosphorus fractions were again measured in samples of this water. The amount of phosphorus eliminated per unit time was determined by difference.

RESULTS

Phosphorus content of mussels

Samples of shell from 7 mussels which had whole shell weights ranging from 0.67 to 55 g had a mean phosphorus content of 0.015% ($s = 0.005$). Samples from 2 smaller mussels which had shells weighing 0.17 and 0.32 g gave values of 0.046% and 0.042%, respectively. The phosphorus in the shell is probably mostly in the conchiolin but some may be in the form of calcium phosphate which will rapidly change to the carbonate during shell formation (Bevelander 1952; Asano and Ito 1956).

The percentage of phosphorus in the body fraction of large mussels changes during the year, being lowest in winter and highest during spawning in September (Fig. 1). The wide range in phosphorus content in the smallest size-class is probably due to differences in size distribution of the mussels within these samples. Bodies which contain more than 1.0% phosphorus are almost always less than 10 mg dry wt. Mussels this small were not present in the September-through-January samples. Analysis of variance indicated that, except in September, the lower percentage of phosphorus in larger mussels is probably a real characteristic (variance ratio less than 10%). Early in September, however, this same characteristic is hidden under the variability caused, presumably, by the release of phosphorus-rich gametes from some individuals. Analysis of variance also indicated (variance ratio less than 1%) that there was a real seasonal change in phosphorus con-

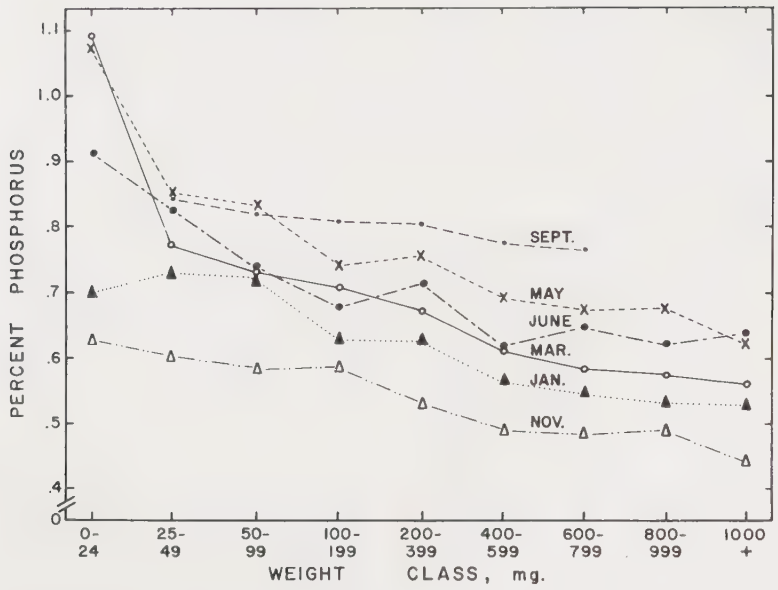

FIG. 1. Mean percentage of phosphorus in the body fraction of *Modiolus*.

tent of mussels of body weight greater than 100 mg.

Since the liquor fraction contained both body fluids and sea water, the dry weight of the liquor varied with variations in sea water salinity. The phosphorus content of the liquor, therefore, was expressed as a percentage of body weight. The mean value of 23 samples was 0.029% ($s = 0.006$). The mean phosphorus content of 8 samples of byssus from mussels growing in beakers in the marsh was 0.05% ($s = 0.006$).

From the data on population density (Kuenzler 1961), the amount of phosphorus incorporated in the mussel population was estimated. Using 4.1 g/m^2 as the mean density and 0.62% as the weighted mean phosphorus content of all classes throughout the year, one obtains 25 mg P/m^2 present in the body fraction. The approximate mean shell weight of 70 g/m^2 times the weighted mean phosphorus content of 0.015% gives about 11 mg P/m^2 in the shells. The liquor fraction, containing phosphorus equal to 0.029% of the body weight, adds another 1.2 mg P/m^2.

The rates of change of the phosphorus in four fractions of the *Modiolus* population (Table 1) were calculated from the recruitment, growth, and mortality rates and the population densities given by Kuenzler (1961) combined with knowledge of the phosphorus content of the various size classes (Fig. 1). The large loss of phosphorus from the body during July and August which is evident in the growth column reflects the loss of weight by the spawning mussels since the phosphorus concentration in the animals was maximal in early September (Fig. 1). From the large positive values, indicating phosphorus accumulation, in May–June and September–October, it would appear that a similar large accumulation took place in July–August, only to be converted to gametes by the end of the spawning period. The total amount of phosphorus released in the gametes or by the gonads, then, might actually be twice as great as the net loss of 4,300 μg/m^2. Since most gametes and larvae perish, this is a real loss by the population. The population lost more phosphorus during the study period than it gained (Table 1).

Phosphorus available to Modiolus

Two experimental feedings showed the maximum particle size ingested by *Modiolus* to be about 160 μ (Table 2). Some of the

TABLE 1. *Changes in phosphorus content of the* Modiolus *population. Values are* $\mu g\ P/m^2$

		Recruitment	Growth	Mortality	Net
BODY					
January–February		5.2	–1,600	–3	–1,600
March–April		9.8	–380	–350	–720
May–June		16	5,900	–870	5,000
July–August		6.2	–4,300	–3,300	–7,600
September–October		13	2,600	–2,700	–80
November–December		2.7	540	–240	300
	Per Year	53	2,800	–7,500	–4,700
SHELL	Per Year	30	1,100	–3,100	–1,900
LIQUOR	Per Year	1.7	130	–350	–220
BYSSUS	Per Year		–160		–160
Total					–7,000

Spartina fragments were irregular in shape so that their maximum dimensions were greater than the pores of the sieves through which they had passed. A few particles in the stomach, therefore, were as much as 1.5 times larger (maximum dimension) than would have been expected (column 2, Table 2). Many particles, large and small, are rejected when mussels are offered a dense suspension of particulate matter, but only small particles are ingested. The minimum size of particles found in the stomach of *Modiolus* was about 3 μ.

The concentration of phosphorus available to mussels in the marsh was estimated from the preliminary data in Table 3. Samples were collected in small creeks, creek heads, or high marsh during flood tide. With the exception of the particulate phosphorus in January through March 1959, measurements of the phosphorus in the three fractions were made by the Hansen and Robinson (1953) method. The estimates for the particulate phosphorus in January through March were calculated from the dry weight of particulate matter per liter on 3 to 6 samples during high tide and the mean percentage of phosphorus. Ten samples were analyzed to obtain the phosphorus content, 0.20% ($s = 0.05$), of this particulate fraction. The data are regarded as only preliminary because a more detailed study of the waters in the vicinity of Sapelo Island is currently being conducted by Dr. L. R. Pomeroy.

TABLE 2. *Particles ingested by* Modiolus demissus

Mussel pair	Sizes of particles fed (μ)	Sizes of particles in stomach (μ)	Quantity in stomach	Quantity of pseudofeces
A	<79	3 to 130, mostly <79	abundant	abundant
B	79 to 164	15 to 240, mostly <150	abundant	abundant
C	164 to 198	10 to 60, mostly centric diatoms	almost empty	abundant; *Spartina* fragments >160 μ

The data concerning the phosphate and dissolved organic fractions are insufficient to permit generalizations beyond their approximate concentrations. In the particulate fraction, however, a downward gradient in concentration is noticeable from the creeks to the high marsh, away from the original water source. Some of this decrease in concentration is attributable to filtration by the mussel population and some to settling of the particles or filtration by other marsh animals. Kuenzler (1961) showed the center of mussel density to be about 220 cm above MLW, *i.e.*, at the same level as the creek heads. Therefore the mean value of 44 μg/L in the creek heads (Table 3) is representative of the amount of particulate phosphorus available to a large fraction of the mussel population. Since samples were taken on both the ebb and flood tides, this value of 44 μg/L was regarded as the mean concentration throughout the tidal cycle over the marsh.

The approximate amount of phosphorus present in the water and ultimately avail-

TABLE 3. *Phosphorus in marsh water. Values are μg P/L*

	Creeks	Creek heads	Short *Spartina* high marsh
Particulate phosphorus			
June 28, 1956[1]	43		
November 28, 1958	47	56	
December 15, 1958	56	26	
January 29, 1959	42	29	27
February 7, 1959	78	58	52
March 26, 1959	84	53	39
Mean	58	44	39
Phosphate phosphorus			
June 28, 1956[1]	25		
December 15, 1958	51	60	
January 29, 1959	46		
Mean	41	60	
Dissolved organic phosphorus			
June 28, 1956[1]	22		
December 15, 1958	14	19	
January 29, 1959	11		
Mean	16	19	

[1] Dr. L. R. Pomeroy, unpublished data.

able to the mussels was calculated by multiplying the mean volume of water inundating the marsh times the concentrations of phosphorus in the water. At 220-cm elevation the mean water depth per covering tide was 21.4 cm; therefore there was about 214 L/m² tide covering the mussel beds. The concentrations of phosphorus present in marsh water at the creek heads (Table 3) were assumed to be the concentrations available to the majority of mussels. At a mean volume of 214 L/m² these concentrations gave values of 9,420, 12,800, and 4,060 μg P/m² present in the three fractions, particulate phosphorus, phosphate, and dissolved organic phosphorus, on the average flood tide. Since there are 1.93 tides per day, 77% of which cover 220-cm elevation, the above values are equivalent to 14,000, 19,000, and 6,040 μg P/m² day, respectively.

Feeding rate

The data from the 16 feeding rate experiments (Fig. 2) show a tendency for a

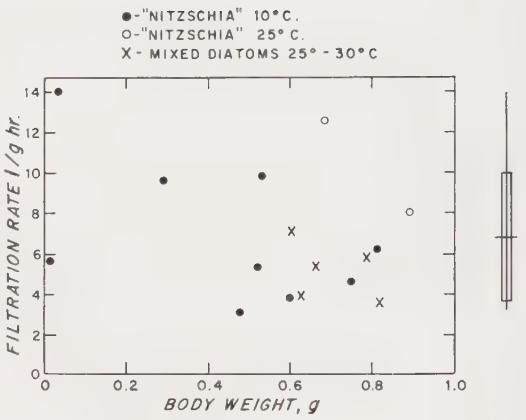

FIG. 2. Filtration rate of *Modiolus*. Shown at the right are the mean, the range, and one standard deviation.

higher feeding rate at higher temperatures. Furthermore, at the same temperature and utilizing the same food, there was some tendency for the smaller mussels to filter at a higher rate per unit weight than the larger ones. However, since the differences due to mussel size, food size, or water temperature were not great, the average filtering rate was determined by lumping all of the data. This mean value is 6.8 ($s = 3.2$) L/g hr; the distribution curve has a positive skew (Fig. 2). The centrifugate of the water in which the mussels were feeding showed that only 3.6% ($s = 2.4$) of the total radioactivity was in soluble form. The centrifugate of the control battery jars, used for determining the diatom settling correction, showed no distinct gain of P^{32} due to loss from the diatoms during the experiment.

Jørgensen (1949) showed a higher feeding rate per gram in small *Mytilus edulis* than in larger individuals. Rice and Smith (1958) showed a decrease in filtering rate with small food particle size or large body size in the clam, *Venus* (= *Mercenaria*) *mercenaria*. They also showed that the filtering rate decreased in very dense (170 cells/mm³) or very thin (0.4 cells/mm³) suspensions of diatoms. With *Modiolus*, in the present study, the density was always above 7 cells/mm³, but 6 experiments were run at densities of 170 to 210 cells/mm³.

There was no obvious decrease at these high densities; in fact, the highest filtration rate occurred at the highest diatom density. An important finding of Rice and Smith was that the rate of filtration by the clam of a labeled natural plankton suspension was about the same as the rate for unialgal suspensions.

Uptake of dissolved P^{32} by *Modiolus* was quite slow compared to uptake of diatom-contained P^{32}. The clearance rates of dissolved phosphate in two experiments were 0.021 (2-hr experiment) and 0.065 2-day experiment) L/g hr, averaging about 0.6% of the rate of filtration of particulate matter. The clearance rate for phosphate in this paper was considered to be 1% that for particulate matter.

Pomeroy and Haskin (1954) reported an uptake rate of phosphate from sea water by oysters of about 4 μg P/oyster day. The phosphate excretion rates of their unfed oysters decreased from about 560 μg P/oyster day for the first 2 days to 20 μg P/oyster day after 33 days. The latter rate was designated the basal excretion rate of phosphate by the oyster, but the former rate is probably more nearly the natural rate. They reported that the oysters might have voided previously accumulated wastes during the first 2 days of the experiment, but it is unlikely that the natural rate was much less than half their reported value. Assuming a natural rate of phosphate excretion by the oyster of 280 μg P/day (half the initial rate) and assuming the oyster eliminates as phosphate about half its total intake of phosphorus (as shown for *Modiolus* below), the total intake would be 560 μg P/oyster day. The uptake rate of phosphate-phosphorus from sea water by the oyster, then, may also be nearly 1% of the total intake of phosphorus. Since not all of the particulate phosphorus which a pelecypod removes from natural water is digested and absorbed, the utilization rate of inorganic phosphorus may be more than 1% of the utilization rate of ingested, natural particulate phosphorus. The efficiency of uptake of phosphorus by mussels feeding on cultured diatoms is considered in the following section.

TABLE 4. *Uptake and elimination of P^{32} by* Modiolus *when fed radioactive diatoms. Values are mean percentages of the loss in radioactivity of the suspension of diatoms*

	Loss in radioactivity (%)	Standard deviation
Body and liquor	34	5
Feces	13	2
Soluble excreta	6.4	1.8
Shell	0.37	0.14
Total	54	

Phosphorus elimination rate

The uptake and elimination of P^{32} by 4 mussels when fed radioactive "Nitzschia" in February are shown in Table 4. A little more than half of the original radioactivity in the suspension was recovered; the remainder was probably lost when the pseudofeces were discarded during washing of the fecal strips. The greatest amount of P^{32}, 34%, was taken up by the combined body and liquor. Feces accounted for 13% of the total uptake from the radioactive suspension in these four experiments, or 11% ($s = 3$) in all 9 experiments where fecal elimination was measured. The high uptake efficiency shown by the ratio of P^{32} in the body to that in the feces is indicative of the digestibility of the diatoms which were fed. Microscopic examination of "Nitzschia" showed that it had very thin frustules, and analyses proved that only 26% of the dry weight was ash. Vinogradov (1953) gives values of from 40% to 80%, usually about 50%, ash in marine diatoms. Hence phosphorus in thick-frustuled natural diatoms may be less easily obtained. Most of the activity found in the shell fraction was on the periostracum surface. This indicated that a large part of the 0.37% uptake by the shell consisted of diatoms which had stuck to the outside and were not completely removed prior to counting or resulted from sorption.

Spawning condition affects retention of phosphorus. Two sexually mature individ-

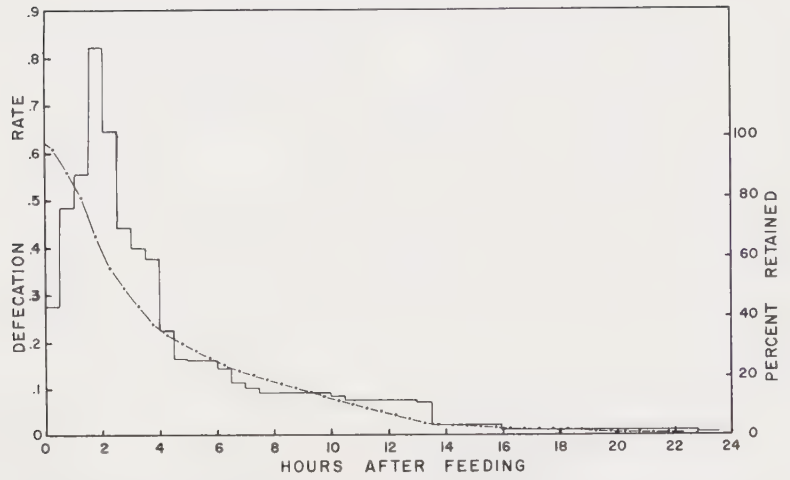

FIG. 3. Time course of defecation. The histogram is the mean fraction of the maximum rate of elimination of labeled feces (scale at left). The dashed curve is the percentage of ingested food remaining in the gut for periods up to 23 hr after feeding (scale at right).

uals, a male and a female, retained amounts of P^{32} in the combined body and liquor equal to 27% and 31% of the loss by the suspension for 21 days after feeding on labeled diatoms. Another mussel which was in the spent condition after being kept in ordinary sea water like the above male and female, through the same dates following a labeled meal, retained only 8% of the loss by the suspension. This is in agreement with other evidence of an accumulation of phosphorus in gametes and loss upon spawning.

The histogram in Figure 3 shows the mean rate of elimination of radioactive feces during each 30-min period after feeding. Since almost all of the diatom suspension was removed during the first half hour, the feeding was considered a very short one, coinciding with only the beginning of the fecal elimination histogram. The maxmium rate occurred about 2 hr after feeding. The dashed curve is an integral of the rates of elimination and depicts the amount of food remaining in the gut at any time after feeding. Assuming that virtually all the ingested food has been eliminated within 24 hr, only 8% remained in the gut as long as 12 hr.

The radioactivity of the body and liquor, the feces, and the shell was measured in the mussel which had taken up dissolved P^{32} for two days. In the feces was found 4.8% of the radioactivity lost by the solution. This P^{32} might have been taken up when the mussel fed on bacteria which had previously removed it from the water, or it might have been taken up by sorption or by bacteria after the faces were eliminated. The body and liquor combined accounted for 31% of the loss from the original solution. The mussel had been in ordinary sea water for 13 hr before it was killed for analysis so any labeled material in the gut should have been largely eliminated (Fig. 3). The shell accounted for 4% of the loss from the original solution, and there seemed to be no difference between the activity of the outside and the inside of the shell. Whether this activity was simply a sorption of P^{32} to the shell or a physiological deposition is not known.

Elimination rates measured by the change in particulate, phosphate, and dissolved organic phosphorus in water containing freshly collected mussels were informative from several standpoints. Analysis of variance showed that elimination rates usually did not vary significantly with temperature. The mean rate of elimination of phosphorus in feces was 56 ($s = 22$) μg/g day (body dry wt), based on a total of 39 experiments

at the three temperatures, 6°, 15°, and 24°C. The mean rate of excretion of phosphate at the three temperatures was 64 ($s = 50$) μg P/g day in a total of 44 experiments. The mean rate of excretion of dissolved organic phosphorus was 5.6 ($s = 5.9$) μg P/g day in a total of 29 experiments at 15° and 24°C. The mean rate of elimination of dissolved organic phosphorus in 9 experiments at 6°C was 33 ($s = 48$) μg/g day. This was not included in the above averages because both analysis of variance and the standard error of difference showed that this rate at 6° does not fit with the values obtained at 15° and 24°C. The reason for a much higher rate of loss of dissolved organic phosphorus at 6° is not known. Temperatures at Sapelo Island are this low for only brief periods during the year, so the error involved in omitting this value is probably not very great.

The large standard deviations are indicative of the variability in rate of phosphorus elimination from experiment to experiment. One cause of differences in excretion rate of phosphate was variation in sea water salinity. Of the 20 phosphate excretion experiments at 24°C, half were performed using water of 12‰ salinity and the other half, 30‰. All of the water was obtained from Doboy Sound, the low salinity water having been collected after a period of heavy rains. The mean excretion rate in the normally saline water was 87 ($s = 54$) μg P/g day, but the rate in the diluted sea water was 13 ($s = 32$) μg P/g day. In spite of the large standard deviations, these rates are significantly different ($P < 0.01$). These data on phosphate excretion at low salinity have been included in the mean for the three temperatures given above because salinities this low or lower were found in 8 out of 79 marsh water samples taken near Sapelo Island by Dr. L. R. Pomeroy (personal communication). Additional work is necessary to determine if salinity has a specific effect upon phosphate excretion or if, perhaps, this is only one consequence of a general osmotic-balance mechanism of salt retention. Kanwisher (1959) showed a pronounced decline in respiratory rate in *Littorina littorea* at salinities below 25‰, so it is possible that decreased phosphorus excretion may simply reflect a lowered metabolic rate in *Modiolus* at low salinities. If phosphate retention during periods of lowered salinity is a general characteristic of euryhaline organisms, it could markedly affect the dynamics of the phosphorus cycle in estuaries.

The salinity differences did not appear to affect the elimination rate of fecal phosphorus. Moreover, since about 92% of the feces from one meal was excreted within 12 hr (Fig. 3), the total amount of feces produced during the experiment really represents only the food ingested during the preceding tide. Since there are two tides per day in nature, the amount of fecal phosphorus eliminated in the marsh is almost certainly twice what was passed in the laboratory. The time course of phosphate excretion indicated a somewhat lower excretion rate after the first day, but the results were not conclusive enough to permit calculation of a correction factor. The amount of phosphate or dissolved organic phosphorus released from the feces by bacterial action after defecation was not measured, but it should be small compared to that released by the mussel.

It is improbable that significant amounts of phosphorus are eliminated in the mucus which binds the pseudofeces together. Any phosphorus so secreted would be a secondary product since mucin does not characteristically contain phosphorus. No analyses were performed, but judging from the ease with which pseudofeces were fragmented, the quantity of mucus in pseudofeces seemed small. Therefore it was assumed that this route of phosphorus loss was negligible.

DISCUSSION

The quantities of phosphorus in the average mussel population (at 220-cm elevation) and in the water over the population, and the routes and rates of flow between these parts of the marsh ecosystem may now be compared (Fig. 4). The body represents only 5% of the total weight of the mussel, but it contains two-thirds of the

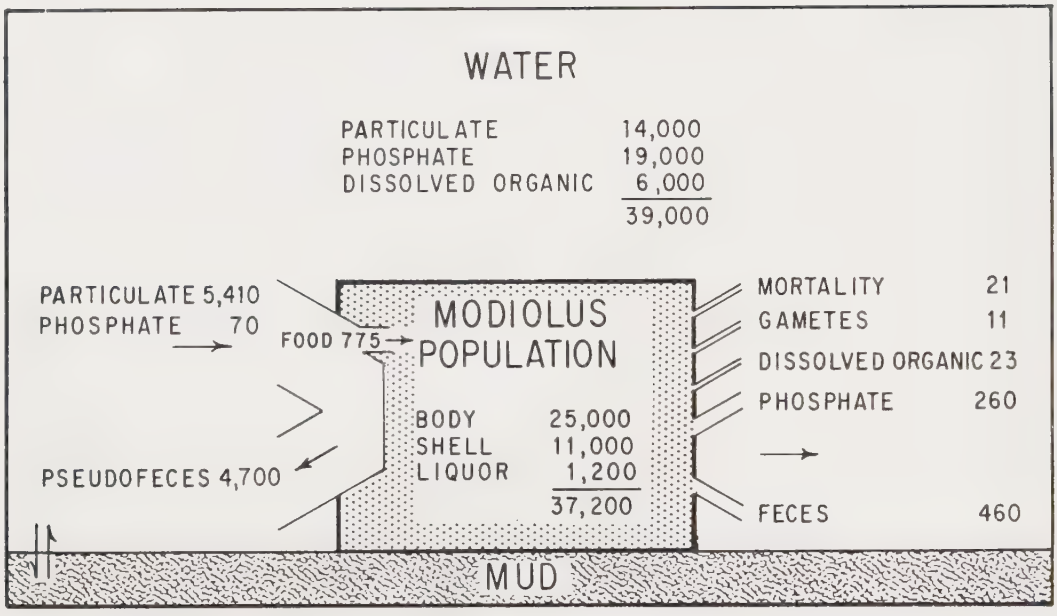

FIG. 4. Diagram of phosphorus flow through the mussel population. Values for the water and the mussel population are μg P/m^2; rates are μg P/m^2 day. The flux rates of phosphorus in food and pseudofeces are calculated values necessary to balance the other, measured flux rates.

phosphorus. The liquor phosphorus amounts to about 3% of the total; the shell contains the rest.

The population phosphorus changes given in Table 1 show that the combined gains by body, shell, and liquor due to recruitment and growth were 4,100 μg P/m^2 yr, whereas the losses due to mortality and byssus production were 11,100 μg P/m^2 yr. It is possible that the population losses were really almost 3 times the growth and recruitment gains during the study period, but it is more probable that the field experiment overestimated the losses and underestimated the gains (Kuenzler 1961). The true values for the population are probably intermediate between the growth and mortality values and should be equal if the population density remains constant. This assumed, intermediate value of 7,600 μg P/m^2 yr, or 21 μg P/m^2 day is shown in Fig. 4 as mortality loss.

The mean elimination rates for fecal, phosphate, and dissolved organic phosphorus were multiplied by the biomass per square meter to obtain the elimination rates per square meter. The rate of loss of phosphorus in feces, doubled to correct for the laboratory underestimation caused by rapid rate of fecal elimination, was 460 μg/m^2 day. The mean rate of phosphate elimination was 260 μg/m^2 day, that of dissolved organic phosphorus was 23 μg/m^2 day. The experimental mussels were not examined thoroughly for disease or parasites, but none appeared to be in poor condition. The commonly occurring thigmotrich protozoans and other small parasites and commensals were undoubtedly present in many mussels; the excretion rates, then, as well as the growth and mortality rates, are those of the mussels with their parasites and commensals.

The amount of phosphorus which leaves the population in gametes during the spawning season was inferred from the decrease in phosphorus content of the population during that season. A minimal estimate, based on the net loss of phosphorus by the population and prorated over the year, was 11 μg P/m^2 day. The true rate may be twice as high for the reasons already given.

The rate of phosphorus ingestion must equal the sum of the rates of its loss from

TABLE 5. *Turnover times of phosphorus in four marine animals*

System	Quantity of P in system	Flux rate	Route	Turnover time, days	Reference
Modiolus population	37 mg/m²	0.32 mg/m² day	Total population losses	115	
Modiolus individuals	6.2 mg/g	0.064 mg/g day	Phosphate excretion	97	
Crassostrea virginica individuals	20 mg/oyster	0.56 mg/oyster day	Phosphate excretion	36	Pomeroy and Haskin 1954
Artemia sp. individuals	—	—	P^{32} uptake	0.58	Harris 1957
Gammarus locusta individuals	—	—	P^{32} uptake	1.79	Harris 1957

the population, or about 775 μg P/m² day (Fig. 4). Since only a little over half the ingested phosphorus is released in feces, the assimilation efficiency for phosphorus is almost 50% in nature. The efficiency of assimilation of phosphorus from "Nitzschia" was higher (Table 4) but this diet cannot be considered representative of the natural one.

The final segment of the cycle in Figure 4, removal of phosphorus from the water, may now be estimated. The rate at which the particulate fraction is removed is the product of the concentration in the water, the rate of filtration, the population density, and the time available for filtration. These four values, 44 μg P/L, 6.8 L/g hr, 4.1 g/m², and 4.4 hr/day, respectively, gave a removal rate of particulate matter equal to 5,410 μg P/m² day. No correction for the decrease in concentration with time was necessary in the calculation since the mean concentration of particulate matter in the water was based upon samples taken on both flood and ebb tides and was, therefore, already corrected for filtration by the mussels. Assuming the removal rate of phosphate to be 1% that of particulate phosphorus, the rate of removal of phosphate by the mussels would be about 70 μg P/m² day. The utilization of dissolved organic phosphorus by mussels has not been proved and is not shown in Figure 4.

From the above calculations, about 4,700 μg P/m² day remains to be deposited as pseudofeces after the 775 μg P/m² day food requirement is satisfied. This large amount of rejected matter is not unusual among pelecypods. Lund (1957), in a study of the feeding of the oyster, found the volume ratio of pseudofeces to feces to be 4.4:1 in natural sea water. The ratio decreased to 2:1 in suspensions which were less dense than natural. Since waters are normally turbid over Georgia salt marshes, filter-feeders could be quite selective in their feeding and reject large quantities of particulate matter.

The turnover time for mussels is longer than those of three other species of marine invertebrates (Table 5). The turnover time for the mussel population, 115 days, is an average for the year. This turnover time may vary seasonally due to changes in feeding, excretion, growth, mortality, and gamete-production rates. The flux rate, 320 μg P/m² day, is the sum of the rates via all pathways out of the population except the feces. Material which has simply passed through the gut has never really entered the body of an animal. Assuming that the fecal phosphorus represents undigested food materials and not true excretion products, it should not affect the turnover time of the population. It probably affects significantly the turnover times of some phases of the water or mud systems, however.

The turnover time for individual mussels might have been estimated with less precision, but with much less effort, by meas-

uring only the phosphorus content of the tissues and the most important flux rate, phosphate excretion (Fig. 4). This gives close agreement with the 115 day estimate, yet it eliminates the laborious field work necessary to determine the flux rates attributable to mortality and gamete production. It may be possible to estimate turnover times for other species and populations by the abbreviated method if the animals and the environments are not too different from those of the present study. It should not be assumed, however, that phosphate excretion is the major pathway of phosphorus out of every population. A population of fast-growing, short-lived organisms might lose proportionately more phosphorus by way of mortality than does *Modiolus*. Larger amounts of phosphorus may be lost in reproductive processes by other populations. Significant losses in other species might be due to secretions or periodic molting. Furthermore, the rate of phosphate excretion might be influenced not only by the metabolism of the animal but also by the availability of phosphorus in the diet; phosphorus may be conserved by animals in a phosphorus-deficient environment. Use of the rate of phosphate excretion alone to estimate phosphorus turnover time may provide a satisfactory estimate for many animals, but a more detailed study will be necessary for many others.

Very few studies of phosphorus turnover in marine animals are found in the literature. Pomeroy and Haskin (1954) did not report turnover time, as such, but gave the data for *Crassostrea virginica* necessary for the calculations (Table 5). Since they reported that the oyster probably voided some previously accumulated wastes at the beginning of the experiment and since allowance cannot be made for phosphorus in tissues other than the body, nor for routes other than phosphate excretion, nor for the effects of storage prior to the experiment, the actual turnover time for oysters may differ somewhat from 36 days. Harris (1957) determined the turnover times for *Artemia* sp. and *Gammarus locusta* to be 14 and 43 hr, respectively, based on the rate of uptake of P^{32} by these crustaceans. His results may be criticized because 1) his formula requires a system with constant precursor activity (Russell 1958), whereas the water in his experimental vessels had decreasing amounts of P^{32} and, presumably, increasing amounts of P^{31} which would cause a lowering of the precursor specific activity; and 2) he showed bacteria to be necessary as intermediaries in the transfer of P^{32} to the crustaceans, and a large part of the measured uptake might have been by bacteria which were on the outside of the animals or undigested in the gut. The phosphorus turnover times of these animals in nature are probably longer than the estimates given by Harris, but they are probably much shorter than those of larger or more slowly metabolizing animals. Good measurements of turnover time for ecologically important crustaceans is desirable since the concentrations of nutrients in aquatic systems may frequently be limited by the rate at which animals can regenerate them.

The mussel population in the salt marsh is only one route of phosphorus into or out of the water. A good measurement of the flux rate by way of this population, however, fixes the maximum turnover time for the water. If the rate of flow of the nutrient into the water by way of another population is determined, this additional value can only serve to decrease the original estimate of turnover time of the system. This will also be true at the population level; discovery of new pathways for a nutrient to, or from, a population can only decrease any former estimate of the turnover time based on the flux to, or from, the population. Conversely, determination of the turnover time for a whole system from knowledge of the rates of appearance or disappearance of a nutrient, for example, sets the maximum flux rate via any one route of the system. Thus, if it were known from the rate of disappearance of P^{32} added to a lake that the turnover time of phosphate in the lake water was four days, then there could be no one population or other component exchanging phosphorus in the lake with a flux rate sufficient to turn over all the

TABLE 6. *Participatory turnover times of phosphorus in sea water due to the* Modiolus *population in the salt marsh*

Phosphorus fraction	Route	Maximum turnover time, days
Particulate	Filtration	2.6
Phosphate	Elimination	73
Dissolved organic	Elimination	260

phosphate in the lake in four days by itself.

These concepts may be succinctly expressed by the following equations:

$$T_t = \frac{Q}{\Sigma F} \quad \text{or:} \quad \frac{1}{T_t} = \sum \frac{1}{T_p}$$

where T_t is the turnover time of a substance in a system; Q is the total quantity of the substance in the system; ΣF is the sum of the flux rates via all routes of the system; and T_p is a participatory turnover time, that is, the turnover time of the substance in the system calculated from any one route. The first equation shows that the estimated turnover time of a whole system will decrease as more and more routes are measured; the second equation shows that the turnover time of the whole system must be less than the turnover time calculated from any participating route.

It is evident that the mussel population has a marked effect on the salt water over the marsh (Table 6). These turnover times for phosphorus in marsh water are participatory turnover times, maximum values calculated under the hypothetical assumption that the mussel population constitutes the only route for particulate phosphorus to leave the water, or for phosphate and dissolved organic phosphorus to enter the water. The biggest effect is the rapid removal of particulate matter from the sea and the deposition of most of it as pseudofeces. The decrease in particulate matter in the sea water with distance from the creeks has already been shown (Table 3) and now the effect of the mussels upon the clearing of the water appears evident. Certainly this is not a permanent loss of phosphorus from the water, but it may account for a significant part of the accumulation of phosphorus and other nutrients in salt marshes. Part of the phosphorus in the pseudofeces and feces is brought back into suspension by water currents, and most of the remainder may eventually return to the water phase as phosphate or dissolved organic matter through the action of important marsh-mud populations such as crabs, snails, polychaetes, nematodes, and bacteria (arrows at lower left of Fig. 4). These marsh populations are all working in parallel and the true turnover times for phosphate and dissolved organic phosphorus in the water phase must be less than 73 and 260 days, respectively (Table 6).

Very little research has been done on marine systems by other workers. Hayes, *et al.* (1952) calculated phosphorus turnover times for two small marine ecosystems from data in the literature. Each system was a rather small salt-water lake which ultimately connected with the sea. The turnover times of dissolved phosphate in the water were 3.2 and 2.4 days based upon the rate of loss of phosphate from the water to lake solids (phytoplankton, mud, etc.) following addition of commercial fertilizer. Bruce and Hood (1959) studied shallow bays and demonstrated that the dissolved phosphate concentration increased at night and decreased in daylight, and that the phosphate was rapidly taken up by the mud. They also reported the turnover rate (*sic*) to be 10 to 20 days, but it is not clear to which compartment or phosphorus fraction this turnover time applies. Pomeroy (1960) used P^{32} to study the phosphate flux between natural suspended matter and water at offshore, estuarine, and marsh stations of the Georgia coast. He found residence times of phosphate to be from 1.7 to 7 days (mean = 3.6) in three samples of salt marsh water near Sapelo Island. Since the particulate phosphorus reservoir is less than half that of the mussel population (Fig. 4), the ability of the plankton population to turn over phosphate in so short a period indicates a flux rate 10 to 100 times as rapid as that of the mussels. It is evident that the suspended matter has by far the greatest effect on the

dissolved phosphate budget; the mussels, in turn, have the major effect on the particulate matter in the water.

In conclusion, the mussels have a relatively long turnover time for phosphorus in spite of an abundance of the element in the surrounding waters. The major fractions of phosphorus present in sea water are ineffectively used (Fig. 4), the phosphate being only slightly utilized and the particulate phosphorus being largely wasted, even after the mussels have filtered it out of suspension. Food requirements other than phosphorus must be satisfied by the filtration process; phosphorus, at least, does not appear to be a limiting nutrient. The mussels, however, have a definite effect upon the water over the marsh, daily removing one-third of the particulate phosphorus from suspension. They regenerate a small part of this into phosphate and reject the remainder in pseudofeces and feces which drop to the mud surface. It appears, therefore, that the mussel population may be very important in the phosphate cycle as a depositional agent, furnishing raw materials to deposit-feeders which regenerate the phosphate. The continual sedimentation effected by the mussels may also play a large part in salt marsh development.

The importance of a population is frequently assessed in terms of its contribution to the entire energy flow of its ecosystem. The total annual energy flow of the mussel population, 56 kcal/m^2 yr, was reported to be about the same as those of the snail, crab, nematode, insect, bird, or mammal populations, but one or two orders of magnitude less than that of the bacteria of the salt marsh (Kuenzler 1961). The rates of water filtration and sediment deposition by the mussels, however, are probably not approached by any other population in the marsh. The mussels, therefore, seem to be more important as biogeochemical agents than as energy consumers in the salt marsh ecosystem.

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BIOASSAY OF ALGAL PRODUCTION IN CHEMICALLY ALTERED WATERS[1]

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ABSTRACT

A bioassy of water from Bear Lake, Idaho, demonstrated that, compared with a nutrient deficiency, the unique chemistry of the water was not a major factor limiting phytoplankton growth. Various chemicals were added to both natural water from Bear Lake and to water chemically simulating it. An alga, *Chlorella vulgaris* Emerson, was used as a bioassay organism. Under the experimental conditions, heavy metal ions added to either imitation or natural Bear Lake waters had no deleterious effect on cell production. Whenever nutrients were present, cell counts increased. A positve correlation existed between algal production and different concentrations of a chelating agent.

INTRODUCTION

The relative importance of water chemistry and nutrient deficiency to algal production was investigated by altering the chemistry of imitation (synthetic) and natural waters of Bear Lake, Idaho. Previous reports (Hazzard 1935 and Kemmerer, *et al.* 1923) have indicated that the standing crop of plankton in Bear Lake is lower than that found in nearby lakes and many other large lakes. Apart from a deficiency in primary nutrients, such as nitrogen and phosphorus, this low plankton production could be caused by the unique chemical composition of the lake.

A water sample from the lake (McConnell, *et al.* 1957), taken in 1952 at a depth of 200 ft and analyzed by the U. S. Department of Agriculture, contained concentrations of elements within the ranges found for its surface water and contained chemicals in parts per million as follows:

Ca	Mg	Na	K	Cl	SO_4	CO_3	HCO_3
17	81	28	6	57	78	18	352

In a survey of northwestern lakes in 1912, Kemmerer, *et al.* (1923) found that Bear Lake had the largest amount of total dissolved solids (1,060 ppm) and a high concentration of zinc (0.65 ppm). The analyses gave far less total dissolved solids (637 ppm) in the 1952 water than in the 1912 water. In 1955, 14 determinations gave an average value of 0.02 ppm of Zn, 0.005 ppm of Cu, and 0.003 ppm of Pb (McConnell, *et al.* 1957). The reduction in Zn is attributed to dilution by Bear River, which is seasonally diverted into the lake for storage purposes.

The chemical characteristics and history of the lake suggest that one or more of several factors may affect its productivity. An ionic complex of the heavy metal ions may inhibit phytoplankton growth to a greater degree than the sum of the effects of individual ions. Because the Mg/Ca ratio is extremely high, excessive quantities of Mg may also inhibit algal production, or the high salt concentration, alone, may have an unfavorable osmotic effect on population growth. Thus, an object of this study was to test the relative importance of these possibilities.

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MATERIALS AND METHODS

Chlorella vulgaris Emerson, strain No. 398, was secured in pure culture from the Culture Collection of the University of Indiana. Stock cultures were grown on nutrient agar, and physiological activity was maintained by frequent transfers to fresh media. The cells used for inoculating the experimental media were grown in liquid cultures.

The basal medium was the same as that found to be optimum for this strain by Pratt (1941). The formula is as follows: KNO_3, 0.03 M; KH_2PO_4, 0.012 M; $MgSO_4 \cdot 7H_2O$, 0.018 M; $FeSO_4 \cdot 7H_2O$, 0.00001 M; and potassium citrate, 0.00001 M.

The salts used were cp grade, dissolved in redistilled water. The trace elements that are necessary for higher plants (Arnon 1938) are apparently present as impurities in these salts in sufficient quantities to permit normal growth.

All media were autoclaved one day before use to insure sterility. The nutrient agar was basal medium plus 15 g/L of solution.

Natural Bear Lake water used in the bioassay tests was obtained in January, 1958, at a position near the deepest part of the lake. The sample contained a mixture of water collected under the ice at various depths down to 50 ft. Because the bottom and surface waters of the lake were mixed during the fall overturn, it is assumed that the chemistry of the sample is representative of the lake as a whole.

Three basic types of artificial solutions were formulated for experimental purposes: imitation Bear Lake water, imitation Bear Lake water without Mg, and imitation Bear Lake water with about equal concentrations of Ca and Mg. The milliequivalents per liter of the chemicals used in the composition of these solutions (Table 1) were calculated directly from the Bear Lake data given in the introduction. Potassium was not included because more than enough was added with 1 ml of inoculate. To help dissolve all the Ca, an excess of CO_2 was bubbled through the solutions that contained no Mg.

The three synthetic solutions, as well as the natural Bear Lake water, were further modified according to the description of the solutions listed in Tables 2 and 3.

Except for the optimum nutrient solution, 0.300 g of KNO_3 and 0.100 g of KH_2PO_4 per liter were added to all solutions where nutrients are indicated in the tables. For solutions containing heavy metals, Zn, Cu, and Pb were added to give concentrations of 0.020, 0.005, and 0.003 ppm, respectively, values the same as those mentioned by McConnell, *et al.* (1957).

Because autoclaving caused salts to precipitate, the solutions were sterilized by passing them through a millipore filter attached to a vacuum pump.

All solutions were placed in sterile vessels of pyrex glass. Subcultures for inoculation were grown in 500-ml Erlenmeyer flasks; experimental cultures were grown in 125-ml Erlenmeyer flasks, each containing 75 ml of solution.

Two separate lots of 24 experimental flasks were placed in a clear glass-bottomed aquarium approximately $30 \times 14 \times 14$ in. in size. In the first lot (Table 1), Solutions 1 to 8 were tested in triplicate flasks and cell counts were made on the 29th, 36th, and 43rd day after inoculation. In the second lot (Table 2), cell counts were made on the 12th, 17th, 24th, 31st, and 38th day after inoculation. Solutions 9 to 17 were tested in duplicate flasks, but Solutions 18 to 23 were not.

The arithmetic means and standard deviations of the temperatures of the first and second lots were 22.0 ± 3.0°C and $23.4 \pm$

TABLE 1. *The chemical composition of synthetic culture solutions of Bear Lake water*

Compound	Normal (Solution 3) meq/L	With Mg equal to Ca (Solution 6 without nutrients) meq/L	Without Mg (Solution 7 without nutrients) meq/L
$NaHCO_3$	0.62	0.62	0.62
$Na_2CO_3 \cdot 7H_2O$	0.60	0.60	0.60
$CaCl_2$	0.85	1.61	1.61
$CaSO_4 \cdot 2H_2O$		1.63	1.63
$CaCO_3$			3.51
$MgCl_2 \cdot 6H_2O$	0.76		
$MgSO_4 \cdot 7H_2O$	3.63	0.85	
$3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	2.36	2.66	

3.1°C, respectively. Excessive fluctuations in temperature were minimized by means of a cooling unit. Illumination for the cultures was supplied by two 40-w warm white fluorescent lamps placed about 18 in. beneath the culture flasks. The illumination was adjusted to approximately 100 ft-c and was constant for 24 hr each day.

The cultures were aerated both to supply CO_2 and to agitate the media. A drying tube containing cotton wool was placed in the air line to filter out bacteria and oil that may have been in the incoming air. The aeration apparatus, with the exception of the pump, was autoclaved to help insure against contamination.

A culture of the inoculum was grown for a number of days until it reached the exponential phase. About 7×10^5 cells/mm^3 were present in the inoculum of the first lot; no counts were made for the second lot. One milliliter of culture was then used to inoculate each of the experimental flasks.

Population growth was estimated by sampling the cultures. Each flask was shaken to obtain an homogeneous mixture and a drop of solution was placed on a haemocytometer. The cells from four areas of the haemocytometer were counted under high magnification after which the counts were converted to numbers of cells per cubic millimeter.

RESULTS

Except for Solutions 1 and 3, a general increase in the number of cells occurred on successive dates. Although there were marked variations in counts among solutions, considerable differences also existed among flasks containing the same solution. An analysis of variance was used to determine whether or not the differences among the solution mean counts were significant in spite of the discrepancies of the flask estimates. Before the data were analyzed, they were transformed into common logarithms.

In Tables 4, 5, and 6 the F-values are equal to 72.68, 3.08, and 141.73 with 7 and 48, 8 and 45, and 5 and 20 df, respectively, and are all significant at the 0.01 level. Consequently, one can assume that the chemistry of the solutions within lots was responsible for the variations in the average size of the algal populations.

To determine which of the solution means were significantly different at the 0.05 level, the "shortest significant range"

TABLE 2. *The logarithmic means of cells of* Chlorella *counted in experimental solutions of synthetic and natural Bear Lake waters modified as indicated and cultured at a mean temperature of 22°C*

Solution number	Description of solution	Cells per mm^3 in logarithms
1	Natural water	3.63
2	Natural water with nutrients	6.08
3	Synthetic water without heavy metals	3.91
4	Synthetic water without heavy metals but with nutrients	4.19
5	Synthetic water with heavy metals and nutrients	5.37
6	Synthetic water without heavy metals but with nutrients and the Mg/Ca ratio equal to unity	5.90
7	Synthetic water without heavy metals and Mg but with nutrients	6.20
8	Optimum nutrient solution	6.90

TABLE 3. *The logarithmic means of cells of* Chlorella *counted in experimental solutions of synthetic and natural Bear Lake waters modified as indicated and cultured at a mean temperature of 23.4°C*

Solution number	Description of solution	Cells per mm^3 in logarithms
9	Natural water with nutrients (same as Solution 2)	6.34
10	90% natural water and 10% distilled water with nutrients	6.46
11	80% natural water and 20% distilled water with nutrients	6.18
12	60% natural water and 40% distilled water with nutrients	6.27
13	Natural water with heavy metals and nutrients	6.53
14	Synthetic water without heavy metals but with nutrients (same as Solution No. 4)	6.17
15	Synthetic water with heavy metals and nutrients (same as Solution No. 5)	5.86
16	Synthetic water with heavy metals and nutrients but with no Mg	6.07
17	Optimum nutrient solution (same as Solution No. 8)	6.73
18	Natural water with 10.0 ppm chelating agent and nutrients	6.70
19	Natural water with 1.0 ppm chelating agent and nutrients	6.39
20	Natural water with 0.1 ppm chelating agent and nutrients	6.17
21	Natural water with 10.0 ppm gibberellic acid	4.74
22	Natural water with 1.0 ppm gibberellic acid	5.20
23	Natural water with 0.1 ppm gibberellic acid	5.03

(SSR) was used (Li 1957). The formula for the SSR simulates that of the "least significant difference" (LSD) and is given as follows:

$$\text{SSR} = ssr \times \sqrt{\frac{s^2}{n}}$$

where s^2 is the error mean square of the analysis of variance; n, the number of replicates; and ssr, the "significant studentized range" for the 0.05 significance level, the values of which are tabulated according to the number of means in a group to be compared and the number of degrees of freedom of the error mean square.

The SSR permits any two means to be compared without requiring a particular solution being designated, *a priori*, as a standard, whereas the LSD requires reference to a designated solution.

A summary of the conclusions obtained by the SSR method of analysis is given at the bottom of Tables 4, 5, and 6. Each solution mean in Table 4 is the average of the logarithms of 9 observations; in Table 5, 10 observations; and in Table 6, 5 observations. The lines under the means link together all those means which are not significantly different from each other at the 0.05 level.

Synthetic Bear Lake water variously modified was used several times throughout the experiment. Synthetic water (Solution 3) was tested to determine whether

TABLE 4. *Analysis of variance and summary of the shortest significant range between logarithmic means of Solutions 1 to 8*

Source of variation	Degrees of freedom	Variance
Solutions	7	13.22127
Days	2	0.71345
Solutions × Days	14	0.21514
Error	48	0.18192

For solutions: $F = 13.22127/0.18192 = 72.68$

Solutions	1	3	4	5	6	2	7	8
Means	3.63	3.91	4.19	5.37	5.90	6.08	6.20	6.90

The underscored means are not significantly different at the 5% level.

TABLE 5. *Analysis of variance and summary of the shortest significant range between logarithmic means of Solutions 9 to 17*

Source of variation	Degrees of freedom	Variance
Solutions	8	0.67303
Days	4	3.19562
Solutions × Days	32	0.08536
Error	45	0.21853

For solutions: $F = 0.67303/0.21853 = 3.08$

Solutions	15	16	14	11	12	9	10	13	17
Means	5.86	6.07	6.17	6.18	6.27	6.34	6.46	6.53	6.73

The underscored means are not significantly different at the 5% level.

or not its productivity was similar to that of natural water (Solution 1). As indicated by the fact that 3.63 and 3.91 are underscored by the same line in Table 4, the means of Solutions 1 and 3 were not significantly different. In both solutions the cell counts were not much different from what could be calculated by diluting the cell count of the 1-ml inoculate 75 times, *i.e.*, diluted inoculate, 9,300 cells; Solution 1, 8,000 cells; and Solution 3, 9,600 cells. For these two solutions, cell counts were less on the 43rd day of culture than on the 29th day. Consequently, little or no growth could have occurred after the inoculation of the flasks.

With the exception of Solution 4, Table 4 discloses that the logarithmic means of the solutions that contained nutrients were significantly different from the means of those that had no nutrients. Any comparisons involving Solution 4 cannot be justified because the value of the mean obtained is extremely low in relation to those for solutions that contained nutrients. In addition, this mean is also very low in relation to the mean of a duplicate, Solution 14, which is discussed below. There is no significant difference between the means of natural Bear Lake water (Solution 2), and synthetic Bear Lake solutions that contained either no Mg (Solution 7) or a reduced amount (Solution 6), but there is a significant difference between the synthetic Bear Lake water in Solution 5 and in Solutions 6 and 7. As expected, the alga grew best in the optimum nutrient solution, a control solution.

For Solutions 9 to 17, Table 5 reveals that the differences in the logarithmic means of the cell counts for the majority of the solutions tested are non-significant. This is attributed to the presence of nutrients in all solutions.

Solutions 9 and 17 were repetitions of Solutions 2 and 8 in the first lot and were added to the second lot for controls. Inspection of their logarithmic means shows no differences attributable to lots.

Cell counts in Solution 4 (synthetic Bear Lake water without heavy metals but with nutrients) were unexplainably lower than those obtained for any of the solutions in which nutrients were added. Consequently, Solution 4 was repeated in the second lot as Solution 14. Because Solution 5 (syn-

TABLE 6. *Analysis of variance and summary of the shortest significant range between logarithmic means of Solutions 18 to 23*

Source of variation	Degrees of freedom	Variance
Solutions	5	3.32916
Days	4	0.29049
Error	20	0.02349

For solutions: $F = 3.32916/0.02349 = 141.73$

Solutions	21	23	22	20	19	18
Means	4.74	5.03	5.20	6.17	6.39	6.70

The underscored means are not significantly different at the 5% level.

thetic Bear Lake water with heavy metals and nutrients) was to be compared with Solution 4, Solution 5 was also repeated in the second lot as Solution 15. No significant difference between the logarithmic means of the cell counts for Solutions 14 and 15 was demonstrated, nor did the means differ significantly from the mean of natural Bear Lake water to which was added additional heavy metal ions (Solution 13).

For Solutions 9 to 12, which were designed to test the effect of dilution on the productivity of natural Bear Lake water, there are no significant differences among the means. The means, however, are smaller for solutions diluted 20% and 40% than for those diluted 10% or not at all even though the same amount of nutrients was added to all four solutions. Thus, the addition of distilled water was not beneficial; although it might have been expected to be because it reduced heavy metal ion and salt concentrations, which conceivably could have had inhibitory effects on cell populations.

The effect of Mg on cell production was tested in Solutions 15 and 16, synthetic solutions to which were added both nutrients and heavy metals. The mean of Solution 15, which contained Mg, was not significantly different from that of Solution 16, which did not.

The mean of the optimum nutrient solution (Solution 17) has the highest value but is not significantly different from the mean of Solution 9 (natural Bear Lake water with nutrients) nor from the means of Solutions 10, 12, and 13 (natural Bear Lake water modified in various ways). That the logarithmic means of so many solutions in the second lot showed no significant differences is attributed partly to the presence of nutrients in all flasks and partly to the great variation in cell counts that occurred between duplicate flasks in certain solutions.

Solutions 18 to 23 were designed to test the effect of different concentrations of a chelating agent (a sodium salt of ethylenediamine-tetraacetic acid) and gibberellic acid ($C_{19}H_{22}O_6$) on algal production. Except for two solutions in Table 6, the logarithmic means were all significantly different. In the range tested, the solution that had the highest concentration of chelating agent produced the most cells. Nutrients were added with the chelating agent because chelation could reduce the amount of nutrients available to the cells along with possible growth inhibitory elements.

Gibberellins produce various types of growth responses and may stimulate cell division. No nutrients were added with gibberellic acid because the object of treating the solutions with acid was to test whether or not the alga could better utilize existing nutrients in natural Bear Lake water. Higher counts were obtained in aliquots treated with this acid than in other solutions in which no nutrients were added. Because the values of the control solutions (Solutions 2 and 9; 8 and 17) are so similar, this increase could be significant. The logarithmic means of the solutions in which the chelating agent was added were all significantly higher than those in which gibberellic acid was added; the reason for this is that nutrients were added to the former and not to the latter solutions.

DISCUSSION

The object was not necessarily to obtain absolute values of growth, but to obtain a relative measure of the effectiveness of various treatments on cell productivity. Towards this end, the experiment was designed so that an analysis of variance could be used as a statistical procedure for interpretation of the results. Because each lot was analyzed independently, indeterminate physiological and quantitative differences in inoculum as well as uncontrollable environmental influences such as temperature between lots are not misleading. The relatively large number of counts per treatment mean reduces the standard error of the mean, and consequently, mitigates random error inherent in subsampling and counting.

Under the experimental conditions, the addition of heavy metal ions to synthetic and natural Bear Lake waters had no

deleterious effect on cell production. This suggests that any inhibiting influences were relatively minor and that more critical experimentation would be necessary to discover to what degree the heavy metal ions effect plankton production.

Although a reduction in the quantity of Mg slightly increased algal growth, such insignificant increases indicate that possible inhibition by Mg on cell counts was relatively small, at least when nutrients were present.

Chipman, *et al.* (1958) have demonstrated that the uptake of Zn^{65} by *Nitzschia* cells was not detectable in 24 hr in sea water and concluded that the chelated Zn of the sea water was not available to the cells. Since chelating agents may increase production, further experiments with agents of this type might prove fruitful. The presence of nutrients in these chelated solutions, however, is noted; if chelating agents are to be used, the addition of nutrients may also be necessary.

On the basis of these exploratory experiments, the availability of nutrients appears to be the main factor limiting phytoplankton growth rather than possible inhibitory effects caused by the chemical composition of Bear Lake water. Further experimentation could be undertaken to determine what specific nutrients are limiting and what minimal amounts of these compounds are needed to increase effectively the productivity of Bear Lake. Ultimately, the results of laboratory experiments should be tested in the field, possibly in small, artificially restricted areas of the lake itself.

That this bioassay of Bear Lake water is purely exploratory should be emphasized. Not only is the scope of this experimental work limited, but different results might be expected from different species or strains of algae especially if those native to Bear Lake were used as cultural organisms.

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EFFECT OF CURRENT ON MINERAL UPTAKE AND RESPIRATION BY A FRESH-WATER ALGA[1]

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ABSTRACT

P^{32} uptake and CO_2 evolution by *Oedogonium kurzii* Zeller were compared in the laboratory in still and moving water. An "inherent current demand" by this lotic species was indicated a) by x10 increase in P^{32} uptake at current equivalents of 18 cm/sec; and b) by a 70% increase in respired CO_2 in dark bottles at current equivalents of 15 cm/sec. In a current this demand is satisfied by the creation of a steep diffusion gradient.

INTRODUCTION

Certain species of plants and animals soon die when brought into the laboratory even though kept at a suitable temperature and in well-aerated water from the locality where collected. These are current-inhabiting species such as the river weed (*Podostomum*) and its relatives, water moss (*Fontinalis* sp.), many fresh-water algae including species of *Lemanea, Batrachospermum, Hydrurus, Cladophora, Oedogonium,* and *Spirogyra* (Smith 1950; Blum 1956). Some seaweeds restricted to wave-swept shores should undoubtedly be included also. Among animals the larvae of the black fly (*Simulium*), the stone flies (Plecoptera), as well as certain caddis flies and crustacea are restricted to lotic environments (Welch 1952).

There is good evidence in the literature for an "inherent current demand" by such species, but inadequate evidence for the causal factors. Recently one of us (Whitford 1960) suggested that a current produces a steep diffusion gradient, thereby increasing exchange of materials between an attached, current-inhabiting species and the water. To test this hypothesis we conducted experiments on the uptake of P^{32} and the liberation of CO_2 in darkness by the green alga, *Oedogonium kurzii* Zeller. This species is a coarse filamentous type available in quantity in rapids near our laboratories.

[1] This work was aided by Contract No. AT-(40-1)-2100 with the Division of Biology and Medicine, U. S. Atomic Energy Commission.

PHOSPHORUS UPTAKE

To compare the rate of phosphorus uptake in running and still water, we suspended tufts of freshly collected algae near the periphery of large battery jars in radioactive phosphorus solutions. The jars were 24-cm inside diameter. The tufts were suspended in 5-cm depth of solution 2 cm from the sides. A current of approximately 18 cm/sec was produced in one jar by means of a magnetic stirrer. Wings of aluminum (8 cm long) attached to the magnet produced a uniform current through the tufts of suspended algae. In the control jar, the tufts were suspended in still, but well aerated water. Two liters of phosphorus solution were placed in each jar. Enough sodium phosphate (NaH_2PO_4) was added to distilled water to bring the phosphorus level to that of the stream where the alga was collected. This was to avoid a phosphorus deficit. In the first two tests, the actual concentration was 10^{-7} M to which was added 0.6 μc/L of P^{32}. In tests 3 and 4 the level of P^{32} was reduced to approximately 0.1 μc/L.

Tufts of algae were removed from experimental and control jars at intervals of from 3 to 80 min, thoroughly washed, macerated, spread and dried on planchets under a heat lamp. Counts of radioactivity were made within 3 hr using a Nuclear Chicago D 47 Gas Flow Counter and a model 186 Decade Scaler. Corrections were made for efficiency and background. Since the total volume of solution was large compared to the dry weight of algae in the jar, the solu-

TABLE 1. *Uptake of P^{32} from still water and from a current (18 cm/sec) by* Oedogonium kurzii *Zeller. Samples C7–11 in still water; E7–11 in a current. Solution 10^{-7} moles NaH_2PO_4 + 0.1 μc/L P^{32}. Temperature at start 29°C; at end of one hour 29.5°C*

Sample	Exposure time (min)	Count/min	Dry weight sample (g)	Count/min/g
E7	5	194	0.0177	10,950
E8	10	606	0.0435	13,930
E9	20	340	0.0119	28,550
E10	30	755	0.0179	42,190
E11	60	447	0.0066	67,800
C7	5	96	0.0493	1,948
C8	10	154	0.0733	2,105
C9	20	148	0.1004	1,479
C10	30	130	0.1647	789
C11	60	255	0.0829	3,079

tion was not aerated during the tests except in one case. Aeration of the solution in the control jar by means of a fine air breaker produced no significant change in results obtained.

Uptake of phosphorus as indicated by counts of radioactivity was significantly greater in the current than in still water. In each of 4 runs an average of 5 time periods were used giving 20 samples from the current and 20 from still water. There were about three replications of each time period. The average phosphorus uptake in the current was 10.7 times that in still water (Table 1). In all tests, phosphorus uptake was inversely proportional to the sample size.

RESPIRATION

Relative rates of respiration in moving and still water were determined for the above species, using CO_2 evolution as a measure of respiratory rate. Single tufts of algae were suspended in 50 cc of stream water in 125-cc sealed flasks. Flasks were covered with aluminum foil to exclude light. Stream-water blanks and algae in still water were stood on the laboratory table and another set was placed on a rotary shaker which produced a steady current past the tuft of algae. The current speed was estimated at 15 cm/sec. The apparatus and method of determining CO_2 were those used by Elkan and Moore (1960) to measure respiration of microbes

TABLE 2. *Respiration in the dark, as determined by CO_2 liberation, of* Oedogonium kurzii *Zeller. Samples C1–2 in still water; E1–2 in a current of 15 cm/sec. Samples in filtered stream water. Temperature 24.5°C. Duration of test 1 hr*

Sample	Dry wt sample (g)	Relative rate of respiration per gram
E1	0.0510	20
E2	0.0561	22
C1	0.0708	11
C2	0.0777	12

in soils. Our only innovations were the use of a rotary shaker to produce a current and a cover to the flasks to prevent photosynthesis during the test. Most tests were run in early morning on algae which had stood in the dark overnight. Realizing, however, that near exhaustion of food reserves or the accumulation of CO_2 overnight might inhibit respiration, we ran one test on algae which had been exposed to light in well-aerated water.

The rate of respiration averaged 70% greater in the current as compared with still water, and it was significantly higher in every run. In the case of the run with algae which had been exposed to light before the test, respiration was 100% higher in the current (Table 2).

DISCUSSION

The results indicate that when a current is greater than 15 cm/sec it produces a steeper diffusion gradient around attached organisms. As a result of this effect, the uptake of vital substances is increased substantially. The experiments also indicate that small variations in conditions and different treatment of collected material before the experiments can have considerable effect on the results. The size of samples used varied because it was impossible to weigh or otherwise measure material without using methods which we believed would damage or otherwise affect the algae. In all cases there was a higher uptake of phosphorus per gram, or a higher respiratory rate, by small samples. This can be explained on the basis of the relatively greater exposure of filament surface to cur-

rent by the smaller samples. If this explanation is correct it is further proof of the current effect. Previous work seems to indicate that the current demand is in part due to a higher respiratory rate in lotic species (Fox, *et al.* 1935; Washburn 1936). We hope to test this hypothesis by using lenitic as well as lotic species in future experiments.

SUMMARY

Proof is offered that the cause of the "inherent current demand" by species of lotic organisms is the need for rapid exchange of materials with the water, and that the steep diffusion gradient in a current satisfies this demand.

Experiments on the filamentous green alga, *Oedogonium kurzii* Zeller, indicate phosphorus uptake is over 10 times greater in a current of 18 cm/sec, and that respiration is over 70% greater than in still water.

Living material should be handled with care to prevent variation in the results obtained.

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MECHANISM OF RADIOACTIVE ZINC UPTAKE BY *ULVA LACTUCA*[1]

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ABSTRACT

The effects of metabolism, pH, carrier ions, and temperature upon the uptake and accumulation of Zn^{65} by *Ulva lactuca* from sea water in light and dark were investigated. The pattern of uptake in relation to light and temperature in unbuffered sea water was what would be expected assuming zinc absorption to be a metabolic accumulation process proportional to the rate of photosynthesis as has been previously reported. However, uptake rate and the level of accumulation was found to be strongly pH dependent and to be affected by the concentration of zinc in the media. Freshly killed *Ulva* absorbed more tracer than live material. These findings suggest that the physical process of adsorption or cation exchange is primarily responsible for Zn^{65} uptake. The relationship between photosynthesis and zinc absorption is primarily a secondary effect related to surface/volume ratio and pH.

INTRODUCTION

Previous studies of zinc uptake by algae have been conducted by Knauss and Porter (1954), who found that the amount of Zn^{65} absorbed by *Chlorella* cultures varied with the concentration of zinc in the media. Rice (1958) reported a rapid and almost complete absorption of Zn^{65} by *Nitzschia* cultures in both the light and the dark, with resulting cell concentration factors up to 50,000. Bachmann and Odum (1960), using 6 species of marine benthic algae, found a direct relationship between photosynthesis and Zn^{65} uptake in the light with no appreciable absorption occurring in the dark. An apparently similar difference between light and dark cation absorption was reported by Scott (1954), using several species of marine algae and Cs^{137}. The question remains whether the difference between light and dark Zn^{65} uptake is the direct result of metabolism or whether it is the result of physical and chemical changes in the environment induced by the metabolism of the plant.

The apparent uptake of any element by an organism, as pointed out by Spooner (1948), can be generally explained by one, or a combination, of three main processes: adsorption, ionic exchange, and accumulation. The process of adsorption involves a concentration of solute ions at a solid–solvent interface, and may go to the extent of removing all but a trace of the solute, depending upon the degree of previous saturation of the surface with the element. The phenomenon of ionic exchange, however, is quite different, for an organism may often maintain an exchangeable internal concentration of an element in addition to that which may be concentrated on the solid surfaces. Accumulation resembles adsorption in that it involves active removal of the element from solution but differs in that the ions may be inaccessibly bound as they are utilized by the cells. The term "absorption" is used, in this discussion, to denote any uptake reaction between a solid and a dissolved substance.

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METHODS

Two principal methods of measuring zinc uptake were employed. The first, an indi-

[1] This study was carried out at the Marine Biological Laboratory, Woods Hole, during the summer of 1960, as part of the training program in Marine Ecology supported by a grant from the National Institute of Health.

rect method, involved measuring changes in the Zn^{65} activity of water samples, thus interpreting a decrease in activity of the water as uptake by the algae. To obtain simultaneously a series of oxygen, pH and Zn^{65} measurements, a single thallus of freshly collected *Ulva* was suspended in a 1,000-ml filter flask containing sea water which was mixed slowly by means of a magnetic stirrer. The water was obtained from the Woods Hole harbor where *Ulva* and other seaweed grew naturally. Illumination up to 600 ft-c was supplied by fluorescent tubes and temperatures were maintained at 22°C. Water samples were withdrawn through the stopper with a hypodermic syringe. So that no bubbles would be introduced, the displaced sample volume was replaced simultaneously through the side tube from a bottle suspended above. The employment of the micro-Winkler method facilitated the removal of 14 consecutive samples for oxygen, pH, and Zn^{65} determinations while displacing less than 10% of the original water volume.

In the second method the radioactivity in the algae was measured directly. Small discs of *Ulva* (3.1 cm^2) were punched out with cork borers, fixed to glass rods with small rings of wax, and placed in flasks of isotope solution. In this way the discs could be removed periodically from the radioactive sea water, blotted lightly, lowered into cellulose centrifuge tubes, counted immediately in a scintillation well counter, and replaced in the isotope solution. Since the adsorption of added zinc on the apparatus was found to be at least partly associated with the presence of bacteria on the surface, 50 mg/L of penicillin were added to the radioactive sea water which had been previously millipore filtered.

The Zn^{65} tracer solution was obtained from Oak Ridge National Laboratory on June 10, 1960, as "Zn-65-P Processed, High Specific Activity," at which time it had a specific activity of 11,766 mc/g. The concentration of Zn^{65} in the media was about 8 μc/L, with various amounts of carrier zinc added where specified. Radioactivity in all

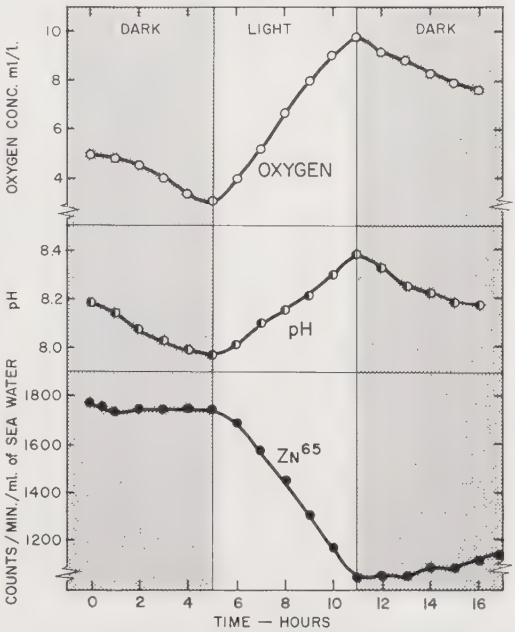

FIG. 1. Changes in pH, dissolved oxygen, and Zn^{65} activity during successive light and dark periods. Initial conditions: 8 μc of Zn^{65} in 1200-ml filter flask of unbuffered sea water, temperature 22.5°C, 0.59 g *Ulva lactuca* (dry wt).

experiments was measured by means of a gamma scintillation well detector connected to an integrating decade scaler. The concentration of zinc is expressed as micrograms per liter.

RESULTS

The curves shown in Figure 1 can be considered typical of four experiments in which simultaneous measurements of oxygen concentration, pH, and radioactivity of water samples were carried out using the indirect determination method of measuring Zn^{65} uptake. In the dark there was very little absorption of added isotope, while in the light the Zn^{65} activity of the water decreased rapidly, indicating uptake by the algae. As would be expected, the pH in the closed system varied with alternating periods of photosynthesis and respiration. Oxygen production in control light bottles showed that the concentration of zinc added with the tracer did not effect the photosynthetic rate of *Ulva*. At a zinc con-

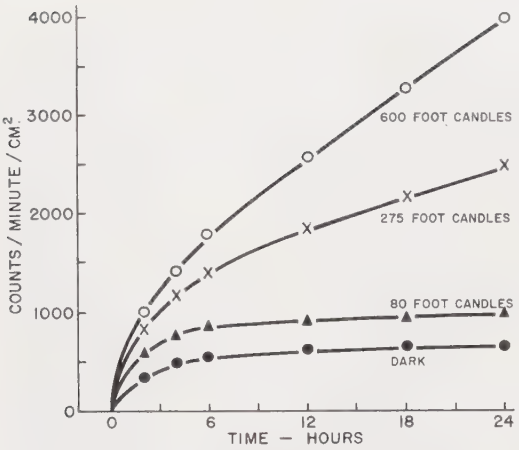

FIG. 2. Effect of light intensity upon Zn^{65} uptake in unbuffered sea water. Initial conditions: 8 μc/L Zn^{65}, 8 μg/L carrier zinc, pH 8.1, temp. 22.5°C.

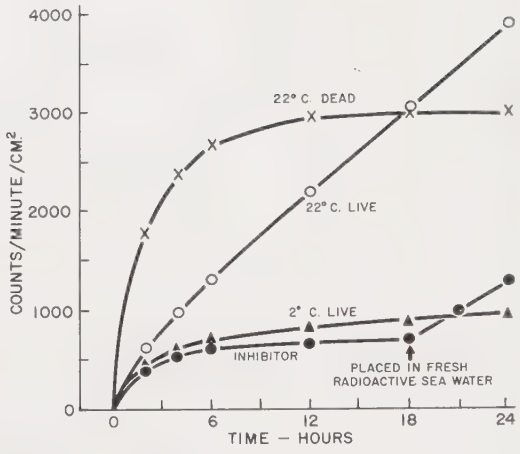

FIG. 3. Effects of a photosynthetic inhibitor, temperature, and killing upon Zn^{65} uptake in unbuffered sea water. Initial conditions: 8μc/L Zn^{65}, 8 μg carrier zinc, pH 8.1, phenylurethane inhibitor 10^{-3} M.

centration of 1,400 μg/L, however, the photosynthetic rate dropped by 25%.

Uptake of Zn^{65} by *Ulva* as measured by the direct, or "disc," method was made at four different light intensities in unbuffered media (Fig. 2). Uptake definitely decreased with decreasing light intensity below 600 ft-c. The compensation point was approximately 80 ft-c, at which level the uptake was slightly greater than in the dark.

To investigate further the relationship between zinc uptake and photosynthesis, an inhibitor, phenylurethane, was added to unbuffered radioactive sea water at a concentration of 10^{-3} M. For comparison, living and dead *Ulva* discs (killed with 3% formalin or steam) were placed in radioactive sea water at temperatures of 2° and 22°C. A control thallus in phenylurathane showed that photosynthesis was effectively inhibited at this concentration. Figure 3 illustrates the resulting concurrent inhibition of Zn^{65} uptake and photosynthesis, followed by at least a partial recovery when the sea water containing inhibitor was replaced with fresh radioactive sea water at the same pH. The effects of temperature upon zinc absorption were pronounced, with uptake being substantially lower at 2°C. Zinc absorption by the killed *Ulva* was considerably more rapid and extensive than by the living material.

Prior to this, the results fitted an assumption that the absorption of zinc by *Ulva* was a metabolic accumulation of ions. However, the rapid uptake of zinc by dead *Ulva* (Fig. 3) raised new questions regarding the actual processes responsible for the observed uptake by the living algae.

The effect of pH upon Zn^{65} absorption was investigated in a series of experiments using living and dead *Ulva* and, for comparison, inorganic particles of Berkshire Grinding Sand. In this series the media were all buffered with Sigma 121 at a concentration of 4.8 g/L, then adjusted to the desired pH with HCl. Figure 4 shows the observed uptake of Zn^{65} by living and dead *Ulva* at several pH's. A pronounced pH dependency of zinc absorption by both living and dead algae is indicated by these curves. A similar pH dependency was found in uptake curves of sand particles. Absorption of Zn^{65} was twice as high at pH 8 as at pH 7 and three times as high at pH 9 as at pH 8. Uptake of Zn^{65} by living *Ulva* at the three controlled pH's was only slightly higher in the light than in the dark.

Three additional experiments were carried out in buffered sea water which contained a cyanide respiratory inhibitor (NaCN at

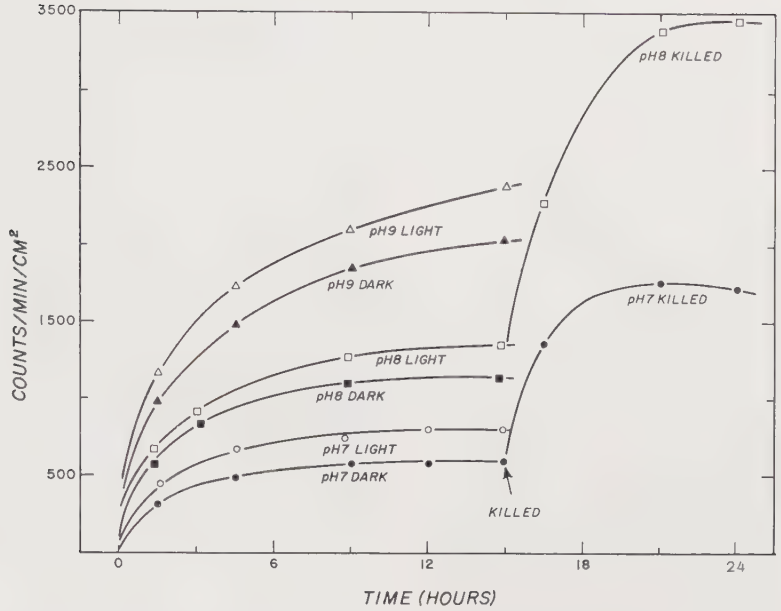

FIG. 4. Effect of pH upon Zn^{65} uptake by living and dead *Ulva*. Initial conditions: 8μc/L Zn^{65}, 8 μg carrier zinc, temp. 22.5°C.

10^{-4} M) as well as the photosynthetic inhibitor (phenylurethane at 10^{-3} M). In previous experiments with dead *Ulva* the discs were killed by immersing in either 3% formalin or steam. It seemed possible that with either of these methods an abnormally large adsorptive surface might have been produced by denaturing the proteins, thus enhancing uptake by the dead tissue. It was hoped that application of both phenylurethane and cyanide would depress metabolism without drastically changing the surface proteins. However, uptake by such poisoned *Ulva*, like the formalin or steam killed thalli, was still much greater than by living thalli at the same pH.

To determine the role of adsorption as a mechanism of Zn^{65} uptake, living *Ulva* discs were exposed to various concentrations of zinc in buffered sea-water solutions. The algal activity at different times was then plotted (Fig. 5) against the equilibrium concentration of zinc in the form of adsorption isotherms:

$$x/m = aC^b \text{ or } \log(x/m) = \log a + b \log C$$

where x is the amount of solute adsorbed, m is the mass of adsorbent, C is the final concentration of the solute in the continuous phase, and a and b are constants. A linear isotherm would have suggested adsorption as the process involved, but in Figure 5 they are all slightly convex. Therefore, some other factor is causing uptake to be slightly less than expected if adsorption were the sole mechanism involved. This could be due to a natural concentration of zinc already in or on the thalli before the experiment.

This possibility was investigated by adding a small number of labeled ions to the naturally occurring zinc ions in the sea-water medium. Assuming initial equilibrium any uptake of labeled ions should here give an indication of the exchangeable zinc present in or on the *Ulva*. Four *Ulva* discs were placed in 300 ml of sea water containing 3 μc of Zn^{65} and buffered at pH 8.1, the same pH as the sea water from which the *Ulva* was collected. The total amount of zinc added was 0.25 μg, increasing the total zinc concentration by an estimated 9%.[2]

[2] The percentage calculation is based on an estimated natural zinc concentration of 9 μg/L, which is the average of 12 values compiled by Richards (1957).

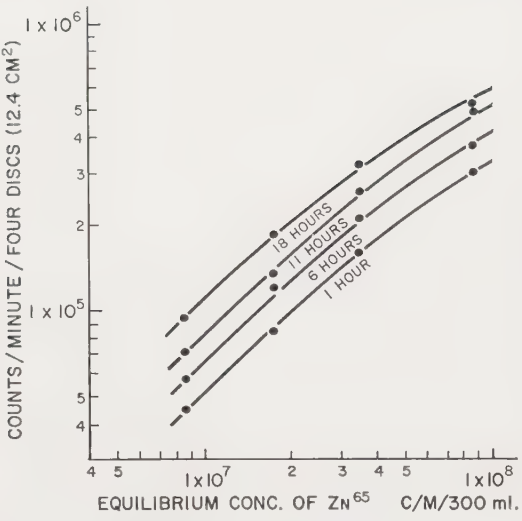

FIG. 5. Adsorption isotherms for Zn^{65} using living *Ulva* in buffered media. Initial conditions: pH 7.5, temp. 22.5°C.

The activity of the algae after 36 hours was 29,000 count/minute, about 4% of the total initial sea-water activity. A concentration factor for the algae, calculated from wt of media × % activity in algae/wet wt of algae × % activity in media, gave a value of approximately 300. The presence of this original concentration of exchangeable zinc may partly expain the convexity of the adsorption isotherms in Figure 5.

Finally, *Ulva* which had been made radioactive in buffered media was placed in a number of buffered wash solutions. As in the case of uptake, pH had a pronounced effect upon the loss of Zn^{65}. After 12 hr, 75% of the activity was lost at pH 6 and 42% at pH 8. Thus, low pH results in greater zinc loss while, as already demonstrated, high pH markedly increases zinc absorption. Several similar experiments using "tagged" dead thalli, although they had taken up over twice as much Zn^{65} as had the live *Ulva*, showed pH dependent desorption similar to the live thalli.

DISCUSSION

The hypothesis that uptake of zinc by *Ulva* is linked to photosynthesis is an attractive one. Experiments at different light intensities and with photosynthetic inhibitors in unbuffered media tend to substantiate the observations of Scott (1954) and of Bachmann and Odum (1960) that this is true for both cesium and zinc. In addition, the effects of temperature upon Zn^{65} absorption make the metabolic accumulation hypothesis seem no less plausible. Later experiments, however, provide evidence that surface adsorption or cation exchange may be the main process involved. The concentration of zinc in the media, pH, and the initial concentration of zinc in and on the algae have been shown to effect the rate of uptake. This is not to imply that these are the only affecting factors, for it seems likely that others, such as surface/volume ratio, chemical composition of the cell and cell wall, and rate of agitation of the media, are also important in determining both the uptake rate and the final concentration factor achieved.

At least two of these factors, pH and surface/volume ratio, can be positively correlated with the rate of photosynthesis. The pH of any unbuffered culture or bottle experiment often reaches or exceeds 9 when photosynthesis is rapid. Odum, Kuenzler, and Blunt (1958) demonstrated a correlation between the surface/volume ratio and the photosynthetic rate in several marine seaweeds including *Ulva*. The apparent correlation, then, between uptake of Zn^{65} and photosynthesis in unbuffered sea water can be at least partly explained by their mutual relationship to these two variables.

A pH dependent factor which has not yet been considered is solubility. The solubility of zinc ions in fresh water has been shown by Carritt (1959) to decrease rapidly with increasing pH in the 7 to 9 range; it falls to about 2 μg/L at pH 9. In sea water, however, solubility of zinc should be somewhat greater due to the formation of complex ions. It seems unlikely, therefore, that the number of zinc ions in solution was limiting in these experiments.

The fact that uptake, and also loss, is pH dependent suggests that Zn^{65} absorption involves cation exchange, *i.e.*, an exchange of zinc ions for hydrogen ions distributed over the exposed surfaces of large insoluble

anions in the algae. Alginic acid and other water insoluble acids occurring in brown algae have been shown by Wasserman (1949) to exist as salts of various metals. Red algae, such as *Porphyra*, also show appreciable cation adsorption which is no doubt due to wall galactan sulfated and uronic acids (Eppley and Blinks 1957). Other common cell components which are more or less effective cation binders are listed by Hutner (1948). The marked tendency of zinc ions to become bound in this manner is further demonstrated by the high uptake of Zn^{65} by dead thalli. This behavior is in contrast to previous reports of experiments with dead algae which showed only slight adsorption of such cations as cesium and strontium (Rice 1956; Williams 1960; Spooner 1949; and Scott 1954).

That the absorptive mechanisms in this study were concluded to be predominantly physical does not imply that this alga possesses no metabolic requirement for zinc, perhaps even in concentrations above those occurring in the environment. Furthermore, it should not be inferred that the physical processes responsible for the removal of zinc ions from sea water are insignificant in enabling the *Ulva* to satisfy this requirement. On the contrary, the ability of the cell wall to bind nutrient ions is probably of vital significance in its ability to compete with other adsorbing substances for these trace elements.

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SOME CALANOID COPEPODS FROM ARIZONA WITH NOTES ON CONGENERIC OCCURRENCES OF *DIAPTOMUS* SPECIES

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ABSTRACT

Seven species of *Diaptomus* are reported from Arizona, including 4 new to the state. *D. shoshone, D. nudus,* and *D. leptopus* occur on the Colorado Plateau; *D. clavipes* and *D. siciloides* are abundant in the mountainous area below the Mogollon Rim; *D. albuquerquensis* occurs rarely in turbid ponds on the Plateau and in the Sonoran Desert; *D. dorsalis* lives in permanent, oligohaline ponds near Phoenix.

Thirty-four examples of congeneric coexistence are presented, including 3 from Arizona. In 24 instances, coexistence involved species of different subgenera. Differences in length of co-occurring species are common and are particularly marked in temporary ponds and saline lakes. Genetic changes in the nature of character displacement may modify dimensions of coexisting species, although environmental factors other than congeneric competition also play a part. Adult diaptomids from Arizona tanks may be larger when early instars are passed during summer rather than winter. Extrinsic factors thus tend to blur morphological character displacement. Modified life cycles and ecological displacement are further intrinsic mechanisms whereby habitats are partitioned, although not revealed by ordinary sampling techniques.

INTRODUCTION

At this time there are only three published accounts of calanoid copepods in Arizona (Marsh 1929; Kincaid 1953; Wilson 1955). These authors reported three species of *Diaptomus* from a total of seven localities within the state. The present paper includes further records of *Diaptomus* from Arizona, based on collections taken between September, 1958, and February, 1961. The collections were made in Coconino, Yavapai, Maricopa, and Gila counties. Thirty-five stations included calanoid copepods. Only a relatively small area of the state has been sampled, but it seems worth while to report preliminary data at this time.

Many fresh-water calanoids show definite geographical, altitudinal, and other ecological distribution patterns. It is not surprising, therefore, that the various physiographic provinces in Arizona are characterized by different copepod faunas. In simplest terms, the Arizona topography includes three main regions. The first is the Colorado Plateau which extends across the northern third of the state, its southern boundary marked abruptly by the Mogollon Rim. The second region is a complex mountainous area, transitional between the Mogollon Rim and the desert below. It extends across the state in a NW–SE direction. The third region is an extensive desert area of fault-block ranges and basins. It occupies the southwest third of Arizona, and is termed the Sonoran Desert in this paper.

The occurrence of congeneric associations in some collections from Arizona stimulated a literature search for other examples of coexistence among species of the genus *Diaptomus*. Several such cases are presented in this paper with notes on the significance of such associations.

I am very much indebted to Mrs. Mildred Stratton Wilson, who, either verified my determinations, or made the original identifications of the Arizona species reported in this paper, and supplied data on the dimensions of certain European diaptomids. I also wish to thank my colleagues, John G. Weise, Norman H. Russell, and Dean Watt for criticizing early drafts of the manuscript.

ANNOTATED LIST

Diaptomus albuquerquensis Herrick 1895

This species has not been reported previously from Arizona, although its range is known to be, "Rocky Mountain States, Utah south into Central America" (Wilson

1959). I have found it in three collections. Two of these were in turbid stock tanks at low altitudes of about 1,100 ft in Maricopa County, where it was associated with *Daphnia similis* Claus. The latter species is said to be restricted to marginal habitats such as temporary ponds and extremely saline waters, where competition and predation are weak (Brooks 1957). The other collection containing this species was taken from a muddy pool impounded by roadfill in Coconino County. The pond was located in the montane forest a few miles north of the Mogollon Rim, at an altitude of about 7,000 ft. *D. albuquerquensis* was not found in collections from the varied mountainous region below the Colorado Plateau and above the Sonoran Desert. It probably occurs in all three regions, however.

An interesting feature, not previously described, was observed in some of the nauplia stages of *D. albuquerquensis*. The furcal setae are asymmetrical, the right one being longer and stouter than the left.

Diaptomus clavipes Schacht 1897

The known range of *D. clavipes* extends from Alberta to Mexico and east to the Mississippi River (Wilson 1959). The earlier papers of Kincaid (1953) and Wilson (1955) contain reports of this species from Arizona.

I have collected *D. clavipes* from 11 stations in Maricopa, Gila, Yavapai, and Coconino counties. It occurs in large impoundments, small turbid stock tanks (including those of a temporary nature) and even, in small numbers, in an old, weed-choked irrigation canal in the Fort McDowell Indian Reservation. Most collections which contained this copepod were taken below the Mogollon Rim in the transitional region above the Sonoran Desert. It did occur, however, in one collection on the Colorado Plateau a few miles north of the Mogollon Rim. Its altitudinal distribution ranged from 1,500 to 7,000 ft above sea level.

Diaptomus dorsalis Marsh 1907

The reported distribution of this copepod is the West Indies and southeastern United States in the Gulf of Mexico region (Wilson 1959). The present report of its occurrence in Maricopa County is the first Arizona record, and marks the western limit of its known range.

D. dorsalis was abundant in collections from four small permanent ponds in the Tempe-Scottsdale-Phoenix region, at an altitude of approximately 1,100 ft. The ponds probably receive their waters ultimately from the Arizona Canal, which flows from the Granite Reef Dam. In some of the ponds *Najas marina* L., a plant typical of salt springs and brackish water, is present. Analyses of waters collected during the winter from one of these ponds showed the chloride content to be 950 mg/L, and the conductivity 1,900 micromhos at 25°C. This chlorinity could be termed oligohaline (Odum 1953).

Diaptomus leptopus Forbes 1882

D. leptopus occurs from the east to the west coast in North America. It is essentially a northern species, ranging south to Oregon on the west coast, and to Virginia on the east coast (Wilson 1959). It has been found throughout the Rocky Mountains, but has never been reported from Arizona. In Colorado, *D. leptopus* has been collected at altitudes from 5,300 to 10,900 ft (Reed and Olive 1958). In other parts of its range it occurs at lower elevations. I have collected *D. leptopus* only from Potato Lake on the Mogollon Rim at the southern edge of Coconino County, between 7,000 and 7,500 ft above sea level. This is a small, shallow body of water, about 12 miles from Pine, Arizona.

Diaptomus nudus Marsh 1904

The reported distribution of this copepod ranges from Alaska south and east to Manitoba, the Hudson Bay region, and down into the Rocky Mountain states (Wilson 1959). It was reported from Arizona by Marsh (1929), Kincaid (1953), and Wilson (1955).

I have found it in 7 locations. These include two ponds on the North Rim of the Grand Canyon National Park, and three

ponds in the Kaibab National Forest north of the Grand Canyon. Apparently one of these was temporary because it contained members of the anostracan genus *Streptocephalus*. Marsh (1929) reported it from Jacob Lake, also in Coconino County and the Kaibab National Forest. Farther south in this county, it was collected in Stoneman Lake, which may have been modified greatly by a weed-eradication program since my sample was taken. It was also present in a roadside pond, probably seasonal, a few miles north of the Mogollon Rim.

D. nudus is, perhaps, one of the most common species in the sinks and shallow lakes of the Colorado Plateau in Arizona, at altitudes ranging from 7,000 to 9,000 ft. However, it is reasonable to assume its presence in ponds below the Mogollon Rim, because Reed and Olive (1958) found it in lakes of the montane, foothills, and plains regions of Colorado.

Diaptomus shoshone Forbes 1893

This high-altitude species of the western states has not been reported previously from Arizona. It was collected in a pool at an altitude of about 9,000 ft in the Kaibab National Forest north of Grand Canyon National Park, in company with *D. nudus*, and *Streptocephalus*. Probably *D. shoshone* is limited to the higher altitudes of the state and possibly to the region north of the Grand Canyon. It may be present in the White Mountains but it is doubtful that it occurs at lower elevations. In Colorado, Reed and Olive (1958) found *D. shoshone* at altitudes from 9,200 to 12,200 ft, but not at lower levels.

Diaptomus siciloides Lilljeborg 1889

This is a widespread calanoid in North America, and has been reported previously from Arizona in collections from Roosevelt Lake, and San Carlos Lake (Kincaid 1953; Wilson 1955). I have found it at 13 stations in Maricopa, Gila, and Yavapai counties at altitudes ranging from 2,000 to 4,500 ft. *D. siciloides* occurs in a variety of habitats, being common in large impoundments and also in stock tanks, including those of a seasonal nature. Its Arizona distribution is very much like that of *D. clavipes*, although not reported yet from the Colorado Plateau. These two species are typical of the mountainous region below the Mogollon Rim, and above the Sonoran Desert.

CONGENERIC OCCURRENCES IN *DIAPTOMUS*

Preliminary assumptions

It is almost axiomatic in zoology that two closely related species, having identical ecological requirements, will be mutually exclusive in restricted habitats. Hutchinson (1951) discussed certain phases of this phenomenon in the Copepoda, and with respect to some vertebrates and other aquatic arthropods, in a later paper (Hutchinson 1959). Also Pennak (1957) wrote of this problem in the copepods and other animals of limnetic communities. On the basis of those papers and other observations, one would expect that congeneric occurrences of *Diaptomus* would be limited to habitats with a variety of niches, and, furthermore, that there would be a difference in the size of the associated species. The latter was pointed out by Hutchinson (1951), who listed a few species of *Diaptomus* which had been reported by several authors as co-occurring.

One might assume *a priori* that small ponds have a paucity of niches when compared to large bodies of water, and that, in general, only one species of *Diaptomus* would be present in, for example, a small stock tank. However, in large bodies of water there would be a greater diversity of niches making it possible for two or more species to exist without coming into direct competition. This would seem reasonable particularly if their food requirements were different, and Hutchinson (1951) suggested that this might be a function of size for filter feeders. This was borne out by Fryer (1954) who found differences between the gut contents of *D. laticeps* Sars, and of the smaller *D. gracilis* Sars in Lake Windemere. Also, Comita and Comita (1957) presented data on the distribution patterns of various instars of *D. siciloides* in a shallow Min-

nesota lake, which indicate that intraspecific competition is greater within one developmental stage than between stages of different sizes. This probably would apply to interspecific competition also.

The effect of a large habitat, presumably with a diversity of niches, is seen in reports from the Great Lakes of North America. Apparently a partitioning of the habitat is relatively easy, and the conditions set forth above for congeneric coexistence do not hold. For example, Davis (1954) reports 5 species of *Diaptomus*, and two other genera of the Calanoida, from the plankton of Lake Erie. Four diaptomids are of the subgenus *Leptodiaptomus*, and one is of the *Skistodiaptomus* group. They are all small forms, and considerable size overlap is present among these species.

Congeneric occurrences in Arizona

In the Arizona collections from small ponds usually only one calanoid was present, but in some of the larger bodies of water at least two species occurred. Furthermore, these simultaneous occurrences involved species of contrasting size and members of different subgenera. In the large impoundments on the Salt River, the association is *D. clavipes* and the smaller *D. siciloides*. This is also true of San Carlos Lake on the Gila River, and Wilson (1955) reported the two species in Mead Lake above Boulder Dam in Nevada. *Diaptomus clavipes* has been assigned to the subgenus *Aglaodiaptomus*, and *D. siciloides* to the subgenus *Leptodiaptomus*. Wilson (1959) gave the length range for males of the former species as 1.28 to 2.2 mm; the males of the latter are from 1.01 to 1.1 mm.

A similar calanoid association in Arizona was noted by Wilson (1955), who reported *D. clavipes* and *D. nudus* from Annex Lake, Coconino Co., and from an undescribed habitat north of Williams. *D. nudus* is a member of *Leptodiaptomus*, and the males are about 1.1 mm long.

D. clavipes and *D. siciloides* are common in collections from the small ponds of Arizona also, but, with two exceptions, they have occurred singly. In a newly impounded tank in Yavapai County both were present. This tank was dry for several months after my first collecting trip, but later both species reappeared. Further developments in this body of water will be watched with interest, because most other comparable habitats contained no more than one diaptomid. Later, the same two species were found in a collection from a somewhat larger stock pond about 4 miles away.

Perhaps such an association in a similar habitat was described by Comita (1951) who found *D. clavipes* and *D. siciloides* in a turbid pond near Alpine, Texas. Moreover, it may be significant that Comita also reported *Cyclops vernalis* Fischer in the pond, because in the Arizona tanks which I have examined, the typical zooplankton community is composed of one diaptomid, one or two daphnids, and one cyclopoid copepod, usually *Cyclops vernalis*.

The only other association of two diaptomids found in Arizona was that of *D.* (*Hesperodiaptomus*) *shoshone* and *D.* (*Leptodiaptomus*) *nudus*. These were in a small, temporary body of water occupying a solution basin in an alpine meadow north of the gate to the North Rim of Grand Canyon National Park. Of the two, *D. nudus* was much more abundant. The size differential between these species is striking; the mean length of *D. shoshone* males in the sample was 3.1 mm which is about 2 mm longer than *D. nudus*.

Congeneric occurrences outside Arizona

Pennak (1957), in discussing zooplankton communities, stated that he found no more than one species of *Diaptomus* at a time in 148 vertical series of plankton-trap samples from 27 Colorado lakes. However, from pre-existing literature records, Pennak cited a few examples of the ". . . uncommon simultaneous occurrences of two species of limnetic copepods. . . ." His paper contains records of 6 different species-pairs of *Diaptomus*, but the list could have been much longer. I wish to present additional refferences to congeneric occurrences of *Diaptomus*. With the Arizona cases mentioned above, there are 34 different combinations

of two or more *Diaptomus* species coexisting. No attempt has been made to survey thoroughly the literature concerning congeneric occurrences of calanoids, but it appears that such occurrences are not particularly rare. However, subgeneric distinctions usually are present in such cases, and size differences between diaptomids of the same community are common.

In Table 1 the 34 congeneric associations are listed, with authors indicated, and lengths of adult males. The measurements are taken mostly from Wilson (1959) except in the rare instances when the lengths were given by individual authors. Some associations which have been published previously have not been included for various reasons. For example, Comita (1956) found *Diaptomus* (*Diaptomus*) *glacialis* Lillj. (2.5–2.8 mm) and *D.* (*Nordodiaptomus*) *alaskaensis* M. S. Wilson (1.44 mm) in a small Arctic lake, but he found just two specimens in one of many collections. Also, there are records of *Acanthodiaptomus denticornis* (Wierz.) (1.5 mm) with *Arctodiaptomus bacillifer* Kölbel (1.2–1.4 mm), and with *Arctodiaptomus laticeps* (Sars) (1.54–1.65 mm) which I have not put in the table, although they are mentioned elsewhere in the text. This is because *Acanthodiaptomus* probably warrants generic distinction, although it is very close to *Diaptomus*. Another thing to emphasize is that several of the species-pairs have been noted by various workers in many locations, and are not particularly uncommon. This is true of *Onychodiaptomus sanguineus* (Forbes), and *Aglaodiaptomus stagnalis* (Forbes) in temporary ponds, for example.

Temporary Ponds and Saline Lakes

In Table 1, the following species associations definitely were reported from temporary ponds; the first pair from Wilson (1953), and from Wilson (1955); the first group of three from Wilson and Moore (1953); and the first four groups from Hutchinson (1951). Others from temporary ponds in Arizona were mentioned earlier: *D. clavipes–D. siciloides*, and *D.*

TABLE 1. *Some co-occurrences of* Diaptomus *species*

Co-occurrences referred to subgenera	Length of males in mm
Herrick (1895)	
Masigdiaptomus albuquerquensis (Herrick)	(0.96–1.5)
Leptodiaptomus novamexicanus (Herrick)	(0.98–1.7)
Bigelow (1923)	
Leptodiaptomus sicilis (Forbes)	(1.1–1.5)
Leptodiaptomus minutus (Lillj.)	(0.9–1.0)
Eddy (1930)	
Leptodiaptomus sicilis	(1.1–1.5)
Leptodiaptomus siciloides (Lillj.)	(1.01–1.1)
Langford (1938)	
Skistodiaptomus oregonensis (Lillj.)	(1.25–1.4)
Leptodiaptomus minutus	(0.9–1.0)
Hoff (1944)	
Skistodiaptomus mississippiensis (Marsh)	(1.1)
Skistodiaptomus pallidus (Herrick)	(1.1)
Hutchinson (citations in, 1951)	
Hemidiaptomus ingens (Gurney)	(5.2)
Diaptomus cyaneus (Gurney)	(3.2)
Hemidiaptomus ingens	(5.2)
Eudiaptomus numidicus (Gurney)	(1.5)
Mixodiaptomus lilljeborgi (DeGuerne & Richard)	(1.5)
Hemidiaptomus brehmi (Mann)	(4.2–5.0)
Arctodiaptomus belgrati (Mann)	(0.98–1.24)
Hemidiaptomus kummerlowei (Mann)	(3.5–4.45)
Arctodiaptomus bacillifer (Kölbel)	(0.87–1.19)
Hesperodiaptomus franciscanus (Lillj.)	(1.1–2.0)
Leptodiaptomus tenuicaudatus (Forbes) (probably *L. sicilis*)	(1.1–1.5)
Eudiaptomus gracilis (Sars)	(1.26–1.35)
Eudiaptomus pusillus (Brady)	(0.98–1.17)
Arctodiaptomus wierzejskii (Richard)	(1.4)
Arctodiaptomus laticeps (Sars)	(1.3)
Tonolli and Tonolli (1951)	
Mixodiaptomus tatricus (Wierz.)	(1.44–1.76)
Eudiaptomus vulgaris (Schmeil)	(1.45)
Comita (1953)	
Leptodiaptomus sicilis	(1.1–1.5)
Hesperodiaptomus nevadensis (Light)	(3.5)

(Continued on following page)

TABLE 1. Continued

Co-occurrences referred to subgenera	Length of males in mm
Ravera (1953)	
Mixodiaptomus laciniatus (Lillj.)	(1.7)
Eudiaptomus vulgaris	(1.45)
Wilson (1953)	
Hesperodiaptomus wardi (Pearse)	(1.24–1.6)
Hesperodiaptomus novemdecimus (M. S. Wilson)	(3.8–4.0)
Hesperodiaptomus kenai (M. S. Wilson)	(1.8–2.5)
Leptodiaptomus tyrelli (Poppe)	(1.1–1.8)
Arctodiaptomus floridanus (Marsh)	(1.0–1.1)
Skistodiaptomus mississippiensis	(1.1)
Aglaodiaptomus marshianus (M. S. Wilson)	(1.3–1.6)
Wilson and Moore (1953)	
Onychodiaptomus sanguineus (Forbes)	(1.0–2.1)
Aglaodiaptomus stagnalis (Forbes)	(3.0–4.0)
Aglaodiaptomus conipedatus (Marsh)	(1.3)
Davis (1954)	
Leptodiaptomus ashlandi (Marsh)	(0.9–1.2)
Leptodiaptomus minutus	(0.9–1.0)
Leptodiaptomus sicilis	(1.1–1.5)
Leptodiaptomus siciloides	(1.01–1.1)
Skistodiaptomus oregonensis	(1.25–1.4)
Fryer (1954)	
Arctodiaptomus laticeps (Sars)	(1.54–1.65)
Eudiaptomus gracilis (Sars)	(1.14–1.23)
Wilson (1954)	
Leptodiaptomus moorei (M. S. Wilson)	(1.15)
Onychodiaptomus virginiensis (Marsh)	(1.24–1.3)
Onychodiaptomus louisianeñsis (M. S. Wilson & Moore)	(1.33)
Leptodiaptomus moorei	(1.15)
Skistodiaptomus pallidus	(1.1)
Wilson (1955)	
Aglaodiaptomus clavipoides (M. S. Wilson)	(2.0–2.3)
Leptodiaptomus moorei	(1.15)
Aglaodiaptomus clavipes (Schacht)	(1.28–2.2)
Mastigdiaptomus albuquerquensis	(0.96–1.5)
Aglaodiaptomus clavipes	(1.28–2.2)
Leptodiaptomus novamexicanus	(0.98–1.7)
Aglaodiaptomus clavipes	(1.28–2.2)
Leptodiaptomus moorei	(1.15)

(Continued in next column)

TABLE 1. Continued

Co-occurrences referred to subgenera	Length of males in mm
Aglaodiaptomus clavipes	(1.28–2.2)
Leptodiaptomus siciloides	(1.01–1.1)
Aglaodiaptomus clavipes	(1.28–2.2)
Leptodiaptomus nudus (Marsh)	(1.1)
Yeatman (1956)	
Skistodiaptomus pallidus	(1.1)
Skistodiaptomus reighardi (Marsh)	(1.0)
Onychodiaptomus sanguineus	(1.0–2.1)
Reed (1958)	
Hesperodiaptomus wilsonae (Reed)	(1.5)
Leptodiaptomus pribilofensis (Juday & Muttkowski)	(1.0–1.6)
Hesperodiaptomus wilsonae	(1.5)
Aglaodiaptomus leptopus (Forbes)	(1.25–2.4)
Leptodiaptomus minutus	(0.9–1.0)
Hesperodiaptomus victoriaensis (Reed)	(2.6)
Arctodiaptomus bacillifer	(1.2–1.4)
Cole (present manuscript)	
Hesperodiaptomus shoshone (Forbes)	(2.59–3.3)
Leptodiaptomus nudus	(1.1)

shoshone–D. nudus. In 6 of these associations there is at least a 2-mm gap in the lengths of the coexisting species. Only one pair from perennial water shows a comparable difference (Comita 1953). It appears from these data that conspicuous size discrepancies are typical of seasonal ponds when two species of *Diaptomus* co-occur, but this is not so pronounced in permanent habitats.

Comita's report is from a highly saline lake, and this may have some significance. In certain respects saline waters resemble temporary aquatic habitats. They have rather specialized flora and fauna, and may be characterized by an absence of certain competitors and predators which occur in other lakes and ponds. This focuses attention on Hutchinson's report (1951) of *Hesperodiaptomus franciscanus*, and *Leptodiaptomus tenuicaudatus* (which I assume is referable to *L. sicilis*) from a playa lake in Nevada. The sizes of these species are shown in Table 1, but were taken from Wilson (1959). Hutchinson spoke of the

"large" *franciscanus*, and the "small" *tenuicaudatus*, although the ranges given by Wilson suggest an overlap in size. Possibly there were greater differences in the mean lengths of specimens in the saline Nevada lake, and they approached the condition typical of the inhabitants of temporary ponds.

In many large, northern lakes, including those of North America, there may be marked differences in the size of limnetic calanoids, but these cases usually involve members of other families — for example, the large temorid *Heterocope septentrionalis* Juday and Muttkowski (3.0–3.8 mm) occurs with smaller diaptomids (*cf.* Hooper 1947).

Coexistence of Species of the Same Subgenus

In Table 1 there are 10 cases where at least two members of the same subgenus occurred simultaneously. In the six examples cited by Bigelow (1923), Hutchinson (1951), Wilson (1953, 1954), and Wilson and Moore (1953) there is no overlap indicated in the lengths of the species involved. Moreover, in the last paper it is not clear to me whether the authors meant all three species are found together at times, or whether *Onychodiaptomus sanguineus* is sometimes found with *Aglaodiaptomus stagnalis*, and at other times with *A. conipedatus*, there being no more than two species simultaneously and never more than one member of the same subgenus. Eddy (1930) reported two species of *Leptodiaptomus* from Reelfoot Lake, Tennessee. There is a slight overlap in the length of these, the largest *siciloides* being about the size of the smallest *sicilis*. Two other instances, described by Hoff (1944) and Yeatman (1956), involved three species of the subgenus *Skistodiaptomus*, which are roughly the same size. The tenth case is that reported by Davis (1954), who collected four species of similar-sized *Leptodiaptomus* in Lake Erie.

Spatial and Temporal Isolation

Among the papers examined in assembling data for Table 1 there was relatively little information concerning differences in the distribution of so-called coexisting diaptomids. Only information found in these papers is presented here, but it must be pointed out that examples of differential vertical stratification, and temporal discontinuity in lacustrine zooplankters are not rare in limnological literature.

Pennak (1957) found no examples of simultaneous occurrences of two species of *Diaptomus* in the Colorado lakes he studied. However, in some of the lakes he found one species at one time of the year, and a second in collections taken at other times. Yeatman (1956) described some spatial and temporal separation for *D. pallidus* and *D. reighardi* within Woods Reservoir, Tennessee. Also, Hoff (1944) found differences in the distribution of *D. mississippiensis* and *D. pallidus* in Reelfoot Lake. Parenthetically, these species may have replaced the two reported earlier by Eddy (1930). The data of Hoff and Yeatman are of particular interest because they were dealing with species-pairs of the same subgenus, and their data suggest that, even though there was coexistence within the lakes, actually the copepods were not together in the strictest sense.

With respect to another congeneric pair in Lake Nipissing, Langford (1938: p. 58) stated, "*D. minutus* differed markedly from *D. oregonensis* in its vertical distribution." Also, Bigelow (1923) found evidence of different vertical distribution of calanoids in Lake Nipigon; *Diaptomus minutus* occurred in the first few meters below the surface, after which *D. sicilis* would extend for a few meters more; *Limnocalanus* was found in strata below *D. sicilis*.

The Possible Role of Character Displacement in Congeneric Coexistence

Differences in subgeneric categories, spatial and temporal distribution, and in body dimensions may permit closely related species to partition and share aquatic habitats. Except for the first difference, such things could be brought about by character displacement in the sense of Brown and Wilson (1956). For example, there may be

genetic extensions and/or restrictions of typical body dimensions when congeneric species are in competition so that there is actually no overlap in such situations. This phenomenon was discussed by Hutchinson (1959) for other arthropods, but earlier (1951) he presented a few of the following data from various authors for diaptomids. *Arctodiaptomus wierzejskii* and *A. laticeps* vary in size, overlapping completely, except in the unusual instances when they co-occur; then small differences in size distinguish them. Thus, the latter averages 1.3 mm when compared to its congeneric associate (1.4 mm) in Loch Moracha. In Lake Windemere, *A. laticeps* ranges in length from 1.54 to 1.65 mm, and its associate, *Eudiaptomus gracilis*, is from 1.14 to 1.23 mm long (Fryer 1954). In Loch Ness, *E. gracilis* is larger (1.26–1.35 mm), but it coexists with *E. pusillus* (0.98–1.17 mm).

In addition to the above examples, there is the case of *D. albuquerquensis* and *D. novamexicanus* reported by Herrick (1895). He described these, as new species, from the reservoir supplying Albuquerque, N. M., and presented lengths for females, which differ from the dimensions given by Wilson (1959). She wrote that the lengths of *albuquerquensis* females range from 1.08 to 1.7 mm, and those of *novamexicanus* from 1.0 to 2.0 mm. However, Herrick's data show no such size overlap for these species where they coexisted. He stated the lengths to be 1.4–1.6 mm, and 1.1–1.2 mm for *albuquerquensis* and *novamexicanus*, respectively.

Thus, there is limited evidence that there are extensions and restrictions of length in some populations, perhaps because of selection pressures in the presence of congeneric competitors. It is not impossible, therefore, that many of the lengths shown in Table 1 reflect more overlap between co-occurring species than is actually the case; very few authors have presented measurements when reporting associated species.

The considerable morphological responses which copepods make to other environmental factors, however, would render questionable anything but carefully controlled comparative data. For example, Tonolli (1949) found mean size values of *Mixodiaptomus laciniatus* in Lago Maggiore to be greater than in representatives of the same species from Charca de Lloroza. He attributed the difference to contrasting mean temperatures in the two bodies of water when early developmental stages occurred. However, in the large Lago Maggiore, *Eudiaptomus vulgaris* coexists with *M. laciniatus*, but probably the latter is alone in Charca de Lloroza, which was described in the following manner: "La cuvetta, molto modesta (12×6 metri di diametro; 35–50 cm di profondità) è ricavata nello spessore di una morena frontale, in terreno calcareo." If this should be the case (Tonolli does not say), an investigator regarding only the presence or absence of a congeneric competitor might attribute the greater growth in Lago Maggiore to character displacement. Also, Ravera and Tonolli (1956) found good correlation in relating body size in 32 populations of *Arctodiaptomus bacillifer*, and 14 populations of *Acanthodiaptomus denticornis*, to the rate of water exchange in the lakes occupied by these copepods. Congeneric associates were not mentioned.

Some preliminary measurements made of *D. clavipes* from Arizona emphasized the complexities of this problem further. Males from one small stock tank, where *D. siciloides* also occurred, had a mean total length of 2.05 mm. In another tank, where *clavipes* had no congeneric associate, the males had a mean length of 2.24 mm. The seasonal factor may have been important in this comparison. The smaller males were collected in February, and the larger individuals were collected in mid-October. Thus earlier developmental stages could have occurred during winter in the first tank, and during summer in the second. Unfortunately most of the Arizona stations were visited only once and there are few data concerning seasonal differences within a single habitat. An exception to this was a desert tank which was sampled on two occasions, and contained *D. albuquerquen-*

sis. The distance from the rostral tip to the base of the caudal setae was measured on adult males from each collection. On 15 March, 1959, they had a mean length of 1.49 mm (range 1.33–1.55 mm). On 15 October of the same year the mean length was 1.64 mm (range 1.48–1.70 mm). Also, two small tanks lying about 0.5 miles apart, both of which contained *D. siciloides*, were sampled at different seasons. On 23 January, 1959, one of these contained adult males with a mean length of 0.97 mm (range 0.85–1.04 mm). The second tank was sampled on 19 September, 1959. At that time the males had a mean length of 1.21 mm (range 1.15–1.33 mm). On the other hand, *D. dorsalis* from two permanent ponds showed no comparable seasonal difference. On 22 January, 1959, one of these populations included males with a mean length of 1.1 mm (range 1.07–1.11 mm). An earlier collection from the other pond made on 10 October, 1958, contained males with a mean length of 1.01 mm (range 0.92–1.11 mm). Perhaps *D. dorsalis* has a longer life cycle in permanent ponds than the other species have in intermittent tanks, and the winter adults are of the same generation as those present in late summer.

Another type of displacement, making possible a sharing of the habitat, could be a modification of the life cycle. Such a thing was reported for *Arctodiaptomus bacillifer*, which has an atypical shortened life cycle in Lago di Monscera, where it lives with *Acanthodiaptomus denticornis.* In this lake it reaches maturity earlier than it does in similar habitats where *denticornis* is absent (Bossone and Tonolli 1954).

Further displacement of an ecological nature would not be revealed by ordinary sampling methods. Certainly a vertical plankton haul from a lake would not indicate the strata occupied by different species of limnetic copepods, and apparent congeneric association might actually be a far cry from the actual case. The data mentioned earlier of Bigelow (1923), Langford (1938), Hoff (1944), Yeatman (1956), and Comita and Comita (1957) suggest this to be true. Other examples of restriction to specific niches could occur within the larger habitat in response to congeneric competition, and yet be overlooked completely without a study of food habits.

Other Isolating Factors and Mechanisms

Coexistence of closely related copepods may be insured through normal intrinsic contrasts not necessarily reflecting character displacement. Differences in their life cycles could be emphasized further by unusual predation and other environmental conditions. Bossone and Tonolli (1954) studied the association of *Acanthodiaptomus denticornis* and *Arctodiaptomus bacillifer* in five lakes. In three of these, the predaceous *Heterocope saliens* Lillj. may have played an important role in reducing the *bacillifer* population at about the time water temperatures were approaching the optimum for *denticornis.* In two of the lakes the water level lowered conspicuously during the summer, bringing about a marked seasonal variation in temperature which favored the differing ecological requirements of each diaptomid.

In Lago Maggiore, Ravera (1955) has shown that *Eudiaptomus vulgaris* and *Mixodiaptomus laciniatus* exhibit different reproductive cycles. The former is the most abundant of the Copepoda in the lake, and has a long life span with polycyclic reproductive behavior throughout the year. By contrast, *M. laciniatus* is a cold-stenothermal, monocyclic species. Many similar examples are to be found in the literature.

It should be noted that among the cyclopoid copepods there are unusual facets of life cycles which may make possible the sharing of limnetic environments by closely related species. The resting stage shown by *Cyclops bicuspidatus thomasi* Forbes (*cf.* Cole 1953) may make it possible for it to coexist with *Mesocyclops edax* (Forbes). The association of the two is common in North America, *Mesocyclops* being abundant in the summer plankton of some lakes at a time when most of the *C. bicuspidatus* are encysted and diapausing in the sediments.

Conclusions Concerning Congeneric Associations

Thus in conclusion, the 34 instances of association between two or more species of *Diaptomus* reported in this paper or summarized from others, usually involve different subgenera, and species of different sizes. In 10 instances there were associations of species of the same subgenus, and in 12 other instances there was a presumed overlap of size between members of different subgenera (Table 1). It should be emphasized, however, that interspecific competition may be less than inferred from such cases; ordinary sampling methods obscure ecological displacement, and without careful study other character displacement and intrinsic mechanisms which partition the habitat may be overlooked.

SUMMARY

Seven species of *Diaptomus* are reported from Arizona. Four of these have not been recorded previously from the state. *D. shoshone*, *D. nudus*, and *D. leptopus* occur in ponds and lakes on the Colorado Plateau; *D. clavipes* and *D. siciloides* are abundant in small stock tanks and large impoundments of the mountainous areas below the Mogollon Rim; *D. albuquerquensis* is uncommon, but was found in turbid ponds above the Mogollon Rim and in the Sonoran Desert; *D. dorsalis* occurs in permanent, oligohaline ponds of the Phoenix area.

Congeneric occurrences of *Diaptomus* species in Arizona habitats, and those reported elsewhere suggest that usually such associations involve different subgenera, and species of different sizes. Some other data are presented, which suggest that interspecific competition may be less than inferred in such associations.

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LABORATORY CULTIVATION OF THE MARINE COPEPOD *PSEUDODIAPTOMUS CORONATUS* WILLIAMS[1,2]

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ABSTRACT

Pseudodiaptomus coronatus Williams was reared at 20°C through 4 consecutive generations, in individual beakers containing 70 cc natural water (20–25‰) using *Chlamydomonas* as food. There were 5 nauplius and 6 copepodite instars. The average generation time was 25 days. Copepodite development was faster in males than in females. The rate of oviposition depended on the presence of males. Females isolated from males after copulation produced up to two viable clutches of eggs.

The ability of copepodites to attach to substrata and the relatively high passive sinking speed of adults may contribute to the spatial distribution of estuarine populations.

INTRODUCTION

Pseudodiaptomus coronatus Williams (Calanoida, Diaptomidae) is a temperate zone representative of a widely distributed, but generally tropical, genus comprising about 30 salt, brackish or fresh-water species (Kiefer 1938). *P. coronatus* lives in the estuaries along the east coast of North America from Nova Scotia (Wilson 1932) to the mouth of the Mississippi River (Wright 1936). Other New World species, with supposedly narrow salinity preferences, are known from South and Central America (Wright 1936); one euryhaline species inhabits the southern west coast of North America (Johnson 1939).

During the summer of 1956, *Pseudodiaptomus coronatus* was reared through 4 consecutive generations at the Marine Institute of the University of Georgia at Sapelo Island, Georgia.

METHODS

Natural brackish water from the tidal Duplin River, Georgia, was used as culture medium. Salinities varied from about 20 to 25‰. The water was decanted from its natural sediment after at least one week's storage in a dark room below 5°C. It was completely clear and contained no appreciable amount of food.

Newborn nauplii reared without the addition of algae were strongly retarded in growth and usually died in an underdeveloped second or third stage.

Chlamydomonas sp., with very few bacterial impurities, served as food. The algae were cultured in bacterium-filtered natural brackish water enriched with $NaNO_3$ and KH_2PO_4. Illumination was constant at about 200 to 300 ft-c, temperature varied from 18 to 22°C. The algae were centrifuged off the enriched medium and suspended in copepod cultures at concentrations varying from about 50,000 to 500,000 cells/cc. The relatively large neonates of *P. coronatus* fed readily on *Chlamydomonas* 20 min after birth, when their stomachs appeared green and parts of the algae could be observed in the intestinal lumen.

Each copepod was reared individually in a 100-cc glass beaker containing approximately 70 cc of the decanted medium. Beakers were washed with distilled water and sterilized before use. As a rule, the conditions of animal, medium, and food were checked twice a day. About every 3 or 5 days, individuals were transferred with a dropper to new medium. When a female had reached the last pre-adult instar, an adult male was added to the culture (for exceptions, see below). Hatched nauplii were transferred individually to new beakers. Temperatures varied from 18 to 22°C. Illumination was artificial and constant at about 50 to 100 ft-c.

COMMENTS ON INSTARS

The species has 11 instars altogether: 5 nauplius and 6 copepodite stages, the last

[1] Contribution No. 27 from the University of Georgia Marine Institute, Sapelo Island, Georgia.

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being the adult form. As in *Pseudodiaptomus euryhalinus* (Johnson 1948), nauplii are asymmetrical in that the right caudal seta is longer than the left one. The degree of asymmetry declines during nauplius development and is apparently absent in copepodites. Davis (1943) made similar observations on *Eurytemora hirundoides* nauplii. In the fifth copepodite instar, the sexes are distinguishable by morphology, size, and molting rate (see below). The adult has been described by Williams (1906) and Wilson (1932). A notable characteristic of the female (maximum length 1.5 mm) is the asymmetrical pair of ovisacs, the right one containing only one or two eggs as a rule. The male (maximum length 1.25 mm) is recognized by the caudal rami and the fifth legs.

DURATION OF DEVELOPMENTAL STAGES

The average duration of total nauplius development, measured from hatching to molting into the first copepodite instar, was 6.2 ± 0.3 days (standard error of mean, $N = 13$; mean temperature of 15 readings 20.4°C). Duration of each of the first 4 nauplius stages was approximately one day and of the fifth about two days. Average copepodite development, measured from the molt into the first copepodite to the molt into the adult, took 12.1 ± 0.6 days ($N = 18$, mean temperature 21.0°C). There was a significant difference ($p < 0.001$) between male and female copepodites from the same clutches: average duration of males ($N = 11$) was 10.5 ± 0.4 days, of females ($N = 7$), 14.6 ± 0.8 days. This difference in molting rate was detectable in the fourth and especially in the fifth copepodite instar. Mean duration from the last molt to the formation of the first ovisacs was 1.9 ± 0.2 days ($N = 12$), from oviposition to hatching, 2.4 ± 0.1 days ($N = 28$). Thus, the completion of one generation at 20°C took on the average $6.2 + 14.6$ (females) $+ 1.9 + 2.4 = 25.1$ or about 25 days.

Temperatures in the natural habitat of the copepod around Sapelo Island were above 20°C from the beginning of May to the middle of October and above 25°C from the end of May to the middle of September. Therefore, 7 or more natural generations may have been accomplished during the period from May to October.

Females exposed continuously to males produced eggs at rather regular intervals. Mean time interval from oviposition to oviposition was 3.2 ± 0.2 days ($N = 20$). The highest number of ovipositions per female was 9. The average number of live nauplii per clutch was 12.5 ± 1 ($N = 19$), the highest number observed was 20. The highest number of live nauplii produced by one female was 123 (9 clutches). The longest life span observed was 57 days (one female). Several individuals, including males, lived over 50 days. Under the described culture conditions, sex ratio was close to unity, with a slight preponderance of males.

The rate of oviposition depended on the presence of males. In several instances, females were raised in the absence of males. In such cases, eggs were produced, but at a significantly lower rate than by females exposed to males ($p < 0.001$): first oviposition occurred 6.1 ± 0.8 days ($N = 8$) after the last molt; subsequent ovipositions occurred at average intervals of 8.4 ± 1.1 days ($N = 5$), the shortest interval observed was 5 days. The egg sacs were of normal size but remained attached for a longer period of time than in the case of fertilized females. Eventually, the ovisacs with the undeveloped eggs were cast off.

Normal nauplii were produced in the absence of males if the females had been previously fertilized. In a few cases, the male was separated from the female after successful copulation, that is, when one to several spermatophores were attached to the female. Such females were subsequently able to produce up to two clutches of normally developing eggs. Eggs were still deposited thereafter, but, like virgin female eggs, did not develop. Presumably, the supply of sperm was exhausted or had become defective.

OBSERVATIONS ON THE BIOLOGY AND ECOLOGY

Mating

The animals are frequently found in copula (*cf.* Sharpe 1910; Johnson 1948). If

isolated male and female groups are brought together, a high percentage of the animals may copulate within a few minutes. Mating attacks of the male may occur under almost any circumstances, even within a glass dropper. Both partners may be swimming or the female may be attached to some substratum (see below). In cultures containing several adults, even copulating pairs were successfully attacked; females with ovisacs and two attached males were no rarity. Males were most frequently observed to attack when they chanced to become situated some 5 mm obliquely behind a female, leaving the impression that the attack might be triggered by the female feeding current. Males were occasionally attacked, but final copulation posture was never achieved.

Following a rather fast approach, the male clasps the female body with its geniculate right first antenna, and tries to attach with its fifth legs to the female urosome. When this is achieved, the antennal grip loosens. In copula, the smaller male hangs more or less in the position of an additional egg sac, facing away from the female, but with its dorsum in the same direction.

Pairs may remain in copula for hours and sometimes for days. In contrast, mating in *Acartia tonsa* Dana was never observed to last longer than a few seconds. In swimming pairs of *P. coronatus*, the female is the more active and leading partner.

Sitting habit and locomotion

P. coronatus is not a true planktonic species since the adults of both sexes are capable of attaching ventrally to substrata, such as the glass wall of the culture vessel, stems of *Spartina*, or larger bits of detritus. In this position, they can withstand considerable currents. Individuals that happened to attach inside the dropper during transfer were difficult to dislodge. The capacity to attach to surfaces arises during copepodite development. Fifth nauplii were never seen resting in this way. First copepodites are able to stick weakly to objects but rarely do so. This faculty of *P. coronatus* may play an important rôle in its vertical and horizontal distribution, especially in strong tidal currents and in regularly flooded *Spartina* marshes.

In the laboratory, adult and fifth copepodite females were usually seen resting on the walls of the culture vessel, whereas males more often tended to swim about near the glass–water interface or in the free water space. Sudden changes in the swimming direction of males were observed with great regularity. Such "searching movements" may serve to increase the chance of finding females. This type of movement was never observed in females.

Vertical distribution and passive sinking speed

P. coronatus has usually been reported in small numbers from surface tows. In the waters near Sapelo Island, the species appears to live close to the bottom, at least during the day. The vertical distribution of *P. coronatus* and *Acartia tonsa* copepodites in Doboy Sound and Duplin River was studied at various diurnal phases, with a Clarke-Bumpus quantitative plankton sampler. In all of 18 series taken, the maximal density of *P. coronatus* was found at the greatest depth sampled, about ½ m above the bottom, which was from 5 to 12 m below the surface. In three cases, another, but at least 50% smaller, density maximum was found from the surface down to 2 m. For comparison, 15 bottom and 13 surface maxima of *A. tonsa* were observed in the same series of samples, the surface maxima being greater than the bottom maxima in 3 out of 12 cases. The greatest observed density of *P. coronatus*, counting all copepodites, was 26,000 animals/m^3. This was taken on 18 May, 1956, in Duplin River mouth at 1830 (mid-ebb tide), about 0.5 m from the bottom in 4.5 m of water at 24.3°C. The corresponding concentration of *A. tonsa* in the same sample was 21,000. During May and June 1956, in certain areas, the average density of *P. coronatus* per water column was often appreciably greater than that of *A. tonsa*.

The common observation that younger stages of planktonic crustacea tend to be closer to the surface than adults can be extended to this species. In 11 cases where

juvenile and adult copepodites were counted separately, the adults only once exhibited a surface maximum in addition to the usual bottom maximum, whereas 5 significant surface maxima were observed for juvenile copepodites.

The causative factors of vertical distribution in *P. coronatus* are poorly understood, but in addition to light, turbidity, turbulence, and internal rhythms, the relatively high sinking speed of the clumsy animal seems likely to be relevant. The following data were obtained by placing live animals in a graduated cylinder (diameter 5 cm), and measuring the time for passive sinking through a given vertical distance. Salinity of the water was about 25‰, temperature was 20°C. The sinking speeds and the standard errors of their means were:

adult males ($N = 31$)	1.30 ± 0.04 mm/sec
females without ovisacs ($N = 33$)	2.32 ± 0.06 mm/sec
females with ovisacs ($N = 35$)	2.78 ± 0.14 mm/sec
copulating pairs with ovisacs ($N = 21$)	3.58 ± 0.15 mm/sec

In contrast, sinking speed was only 0.99 $\pm$ 0.05 mm/sec ($N = 30$) for both sexes of the slender species *A. tonsa*, which lacks ovisacs.

DISCUSSION

The rearing conditions for *P. coronatus* doubtless need further improvement and standardization. The medium was chemically undefined, particularly with respect to organic solubles. It was not bacterium-filtered and there were some bacteria in the algal cultures. Food quantities were not standardized. It is uncertain how close to optimum the employed laboratory conditions were. Mortality was small but not measured quantitatively.

Nevertheless, this species clearly belies the notorious reputation of marine calanoids to defy laboratory cultivation. In fact, it may prove to be a promising laboratory animal. It should invite the study of nutritional requirements, analogous to the work on the harpacticoid *Tigriopus japonicus* (*cf.* Shiraishi and Provasoli 1959). Since mating fervor is easily elicited, the species should be suitable for laboratory analysis of copepod sexual behavior. The generation time being relatively short, genetic problems could be attacked experimentally. *P. coronatus* inhabits the narrow estuarine band from Nova Scotia to the Gulf of Mexico, only a few kilometers wide but some 4,000 km long. Along a drastic gradient of mean temperature, geographical races may almost certainly be expected. The genus as a whole is interesting because of its generic euryhalinity and world-wide distribution. Should closely related species, particularly the South American *P. richardi* and *P. acutus*, prove similarly suitable for laboratory culture, hybridization experiments may attack such problems as salinity requirements, food preferences or behavior. They might well yield results of ecological and evolutionary significance.

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DECONTAMINATION OF FILTERS IN THE C^{14} METHOD OF MEASURING MARINE PHOTOSYNTHESIS

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ABSTRACT

Accepted procedures for decontaminating membrane filters containing radioactive phytoplankton may result in variable and large losses of activity. Results suggest that errors due to decontamination procedures may exceed those which would result from contamination. It is suggested that correction for contamination be made by subtracting the activity of untreated dark blanks from those of untreated illuminated samples.

INTRODUCTION

In the C^{14}-method of measuring marine photosynthesis, it is common practice to expose membrane filters containing the active plankton to hydrochloric acid (HCl) to remove extracellular contamination (Strickland 1960). In the original Nielsen method (1952) the dried filters were exposed to the fumes of concentrated HCl for about 20 min. For greater speed and convenience it is now more common to "suck through" about 10 ml of dilute HCl at the end of sample filtration, although some workers prefer to use isotonic sodium chloride (NaCl) (McAllister, Parsons, and Strickland 1960) or filtered sea water (Thomas and Simmons 1960).

Concentrations of acid used in rinsing filters range from 0.001 N (Ryther and Vaccaro 1954) to 1.0 N (Smith *et al.* 1960) and the literature contains contradictions concerning the efficacy of the various strengths in use. The purpose of this note is to summarize the results of some recent experiments on methods of filter decontamination.

METHODS AND RESULTS

Three hundred milliliter aliquots of a coccolithophore (*Syracosphaera carterae*) culture killed with neutral formalin, and of a suspension of calcium carbonate ($CaCO_3$) in the filtered coccolithophore medium (modified ASP-2) were incubated with 20 μc of C^{14} for 4 hr in the dark. They were then filtered, the filters dried without treatment, and their C^{14} uptake measured. Uptake of radiocarbon by live coccolithophores held in the dark was also measured. The uptake by HA Millipore filters alone was determined after sucking through 300 ml of filtered coccolithophore medium inoculated with 20 μc of carbon.

In similar experiments using the above materials the effects of decontaminating the filters were tested by sucking through three 10-ml rinses of filtered medium; by filtering 10 ml of 0.002 N HCl in 3.5% NaCl followed by two rinses with medium; and by exposure of the filters to the fumes of concentrated HCl for one minute. The activities of the samples were measured in a gas-flow geiger counter, giving about 23×10^6 count/min with a 20 μc source.

The $CaCO_3$ had a "thickness" of about 0.5 mg/cm^2 on the filters, an amount considerably more than that likely to be encountered in practice. The chlorophyll *a* concentration in the coccolithophore culture was 5 mg/m^3. This was high for oceanic waters, but low for waters containing a bloom of phytoplankton. The uptake of activity by dead coccolithophores may be regarded as a *crude* estimate of "non-biological" uptake of radioactivity.

Typical results are shown in Table 1. Bare filters, $CaCO_3$ and dead *S. carterae* retained a very small fraction of the added activity. The live coccolithophores incubated in the dark retained about 15 times the activity taken up by those killed with formalin.

Rinsing with medium removed little of the activity from bare filters, $CaCO_3$ or dead coccolithophores, while exposure to both dilute HCl and acid fumes removed

TABLE. 1. *Decontamination of filters bearing non-living matter*

Method of decontamination	Bare filters			$CACO_3$			Dead coccolithophore			Live coccolithophore		
	count/ min	% of added activity	% loss	count/ min	% of added activity	% loss	count/ min	% of added activity	% loss	count/ min	% of added activity	% loss
None	80	3.5×10^{-4}	0	2,000	8.7×10^{-3}	0	230	1×10^{-3}	0	3,700	1.6×10^{-2}	0
Rinse with medium	90	3.9×10^{-4}	0	2,100	9.1×10^{-3}	0	170	7.4×10^{-3}	27	—	—	–
Rinse with 0.002 N HCl	14	6×10^{-5}	83	150	6.5×10^{-4}	93	5	2.2×10^{-5}	98	—	—	–
Acid fumes	20	8.7×10^{-5}	75	120	5.2×10^{-5}	94	5	2.2×10^{-5}	98	2,880	1.2×10	22

most of it. Exposure to acid released about 80% of the C^{14} from the dead coccolithophores whereas the live cells incubated in the dark showed a 20% loss of activity on fuming and an apparent *increase* with the dilute acid treatment (see below). The loss of activity on exposure of the live dark blanks to acid fumes was about 900 count/min, about three times greater than the activity of the untreated dead coccolithophores.

Centrifugates of cultures of the coccolithophore and a flagellate (*Monochrysis lutheri*) were exposed to isotonic sodium chloride and to 0.002 N HCl in isotonic NaCl. Exposure to isotonic NaCl alone had no visible effects on either the coccolithophore or the flagellate. The $CaCO_3$ platelets of the coccolithophores dissolved immediately on contact with the acid and after 20–30 min exposure 1–5% of the cells showed signs of rupture or collapse. The flagellates stopped swimming on addition of the acid but no further effects were observed during exposures of up to one hour.

The effects of exposure to 0.002 N HCl, HCl fumes, istonic NaCl and culture medium on the activity of the coccolithophore, *S. carterae*, incubated in the presence of C^{14} were determined. Experiments were also carried out with *M. lutheri* and the effects of exposure to 0.002 N HCl on C^{14} activity were measured on a coastal population, on two oceanic populations, and on coccolithophores exposed to radioactivity in the dark. In all cases the samples were inoculated with 1 or 5 μc of C^{14} and illuminated for 4–6 hr at 0.06–0.08 langlies/min of photosynthetically active light.

Results are shown in Figures 1, 2, and 3. The activities of the treated filters are presented relative to those of controls treated with isotonic saline alone.

DISCUSSION

It is evident that activities of treated filters vary with organism, decontaminant, duration of exposure, and in the case of the coccolithophores, with illumination. Decontamination with the fumes of concentrated HCl gave the least variable results.

Losses of 25–30% of the initial control activity seem high, particularly when it is considered that the absolute decrease from control levels exceeded the total uptake by dead coccolithophores by a factor of several times. Also the activity of the initial controls themselves may be as much as 25% less than comparable untreated filters. This suggests that these large decreases are due to loss of intracellular C^{14} rather than to decontamination.

The unexpected increases of apparent activity that are shown could result from decreases in the self-absorption of β radiation by the plankton. If cell breakdown due to suction occurred, the action of the decontaminant might be to spread active matter more evenly over the filter.

Lasker and Holmes (1957) and Guillard and Wangersky (1958) leave little doubt that phytoplankton cells may be damaged by vacuum filtration and that loss of cell contents from this cause may be a serious source of error in C^{14} measurements of marine photosynthesis. They suggest that the degree of injury depends partly on the age of the culture or population and on the degree of clogging of the filter. The extent of injury may further depend on the spe-

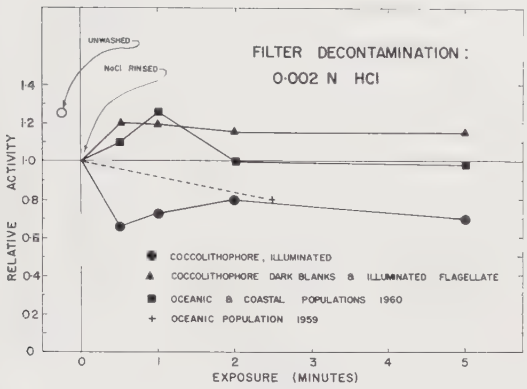

FIGURE 1

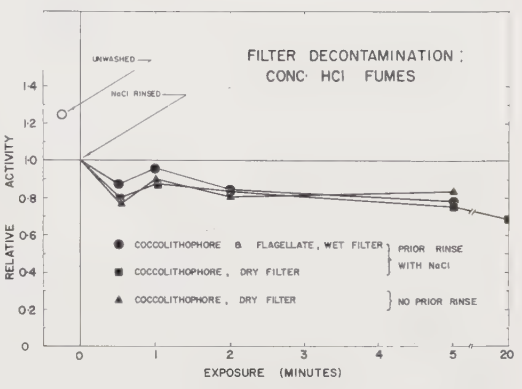

FIGURE 2

cies and doubtless on the nature of the decontaminant and the length of exposure to it.

However, if the large losses of activity from coccolithophores observed in the present work were due mainly to filtration damage, exposure to NaCl and to culture medium should have resulted in similar losses. This was not the case. Exposure to culture medium alone released about 5% of the control activity compared with about 30% with the NaCl, suggesting that NaCl causes losses in addition to those from filtration damage.

In any case, damage due to filtration can only be increased by sucking decontaminant through the filter. This suggests that acid fuming would be a preferable treatment. The results in Figure 2 indicate that fuming does give more consistent results and illuminated coccolithophores did lose less activity with fuming than with the rinsing. However, the reverse was true for the coccolithophore dark blanks and *M. lutheri*. Thus, although fuming does give more consistent results, it may in some cases cause greater losses than acid rinses.

The 20-min fuming recommended by Nielsen (1952) led to a large loss from coccolithophores (Fig. 2). Results in Table 1 suggest that most decontamination of inorganically held activity is accomplished within the first minute of exposure.

The loss of C^{14} from the coccolithophore may be partly attributed to release of activity held in the $CaCO_3$ of the coccoliths. If the presence of activity in the coccoliths is due to inorganic exchange, it could be regarded as contamination. If, however, it is incorporated photosynthetically it could be argued that it represents photosynthetic production in spite of its inorganic nature.

If the C^{14} in the coccoliths is photosynthetically fixed, then dark blanks should lose little activity compared with illuminated samples on exposure to acid. This was the case with 0.002 N HCl wash but losses were similar on exposure to HCl fumes. However, treatment with sodium chloride solution, which does not dissolve the coccoliths (see above), reduced activity by an amount similar to that occurring with rinses with dilute acid, suggesting that only a negligible amount of C^{14} is incorporated into the coccoliths during a 4–5 hr period of incubation.

In view of the variability of results in

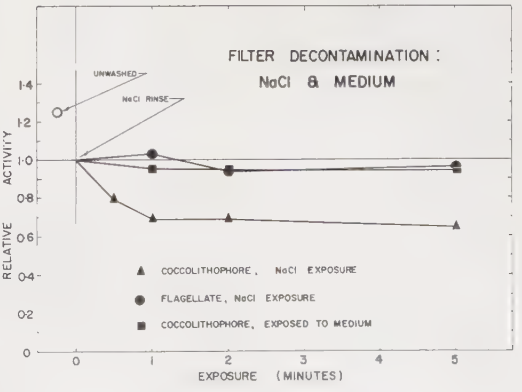

FIGURE 3

even this short series of experiments the confusion in the literature is not surprising. However, the extremely low percentage retention of added activity by bare filters, dead coccolithophores and $CaCO_3$ suggests that contamination of the filters may result in smaller errors than those incurred by the decontamination procedures.

It is suggested that the correction for contamination could be made by subtracting the activities of untreated dark blanks from those of untreated illuminated samples. If rinsing the filter funnel is deemed necessary after filtration, small rinses with sea water or filtered medium would probably cause less error than rinses with NaCl or dilute HCl.

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SCATTERING PROPERTIES OF DISTILLED AND NATURAL WATERS[1]

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ABSTRACT

Measurements of the volume scattering function and other optical properties are presented for distilled water, Pacific coastal and offshore water, and lake water. The depth and time variability of Pacific water at one station is given and the effect of a wind storm on the optical properties of the lake water is demonstrated.

INSTRUMENTATION

The scattering meter used in this work was originally described by Tyler and Richardson (1958). It has recently been critically tested for performance by comparing the results of measurements on prepared stable hydrosols by two independent methods, one of which employed the scattering meter, the other employed an optical integration method described by Tyler (1957). The results of these tests are reported elsewhere.

In order to maintain the accuracy of the data obtained with the scattering meter it has been necessary to maintain its calibration in several respects as follows:

1. *Angle calibration*—The scattering angle θ is related to the output of a voltage divider circuit supplied by a highly stable power source. The phototube arm is geared directly to the potentiometer of the voltage divider which governs the position of the pen of a Brown synchronously driven strip-chart recorder. The performance of this circuit and power supply has been checked at the beginning of every series of measurements and can be post checked for each individual run if desired. The voltage divider has proved to be very precise. The indication of angle is accurate to ± .25° at 180°, ± .13° at 90° and smaller fractions of an angle at 0°, the forward scattering direction. The error in the indication of angular position is generally no larger than the error encountered in reading the data.

2. *Circuit calibration*—The output of the multiplier phototube circuit is designed to be proportional to the log of the light input to the phototube. The circuit used is not quite linear over its 5-log range. Data obtained with this circuit is read by means of a scale which is made by exposing the multiplier phototube to known steps in light

TABLE 1. *Scattering properties of commercial "distilled" water samples. Bandwidth limited by a Wratten No. 57 filter*

Sample	A	B
Total attenuation coefficient α/m	.062	.047
Total scattering coefficient s/m	.00845	.00457
Absorption coefficient a/m	.0536	.0424
Forward scattering coefficient f/m	.00763	.00396
Backward scattering coefficient b/m	.00082	.000620
Ratio f/s	.900	.870
Volume scattering function $\sigma(\theta)$		
$\theta =$ 0°		
10		
20	.00648	.00316
30	.00223	.00107
40	.000941	.000520
50	.000473	.000294
60	.000271	.000191
70	.000181	.000128
80	.000140	.000096
90	.000117	.000083
100	.000110	.000079
110	.000118	.000082
120	.000126	.000092
130	.000134	.000102
140	.000139	.000112
150	.000146	.000119
160	.000171	.000141
170	.000193	.000161
180	.000201	.000169

[1] This research was supported by a grant from the National Science Foundation, Earth Sciences Division. The α-meter, scattering meter, and other instrumentation used in this research were developed by the Visibility Laboratory under a Bureau of Ships contract. The loan of these instruments for the present measurements is gratefully acknowledged.

TABLE 2. *Scattering properties of Pacific Coastal and offshore water at the Stations shown in Figure 1. Bandwidth limited by a Wratten No. 57 filter*

Station number	1	2	3	4
Total attenuation coefficient α/m	.736	.129	.118	.111
Total scattering coefficient s/m	.125	.01094	.01420	.0120
Absorption coefficient a/m	.611	.1181	.1038	.099
Forward scattering coeficient f/m	.119	.01010	.01321	.0110
Backward scattering coefficient b/m	.00630	.000847	.000982	.000984
Ratio f/s	.950	.925	.930	.915
Volume scattering function $\sigma(\theta)$				
$\theta =$ 0°				
10				
20	.1014	.00881	.01192	.00959
30	.0360	.00268	.00358	.00313
40	.0152	.00117	.00145	.00129
50	.00739	.000616	.000698	.000661
60	.00419	.000356	.000396	.000388
70	.00266	.000232	.000253	.000249
80	.00181	.000164	.000179	.000175
90	.00134	.000132	.000145	.000142
100	.00109	.000120	.000134	.000130
110	.000940	.000120	.000135	.000135
120	.000903	.000124	.000146	.000146
130	.000912	.000134	.000158	.000159
140	.000944	.000145	.000175	.000176
150	.001003	.000156	.000192	.000193
160	.001028	.000175	.000202	.000208
170	.001036	.000191	.000206	.000219
180	.001037	.000197	.000207	.000223

level, using the inverse square law to establish the steps. Scales of this kind have been made on the average of every two months during the work, usually just before or just after a series of measurements.

The circuit has ben found to vary ± 2.5%

TABLE 3. *Variability in the optical properties of coastal water at Station 4. Data taken in November 1960. Bandwidth limited by a Wratten No. 57 filter*

Depth (m)	α (per m)	s (per m)	a (per m)	f/s	Time	Tide (ft)
20	.218	.0205	.197	.935	1850	
10	.162	.0176	.144	.940	1910	
1	.148	.0156	.132	.928	1925	4.4
20	.183	.0200	.163	.940	2200	
10	.153	.0173	.136	.935	2213	
1	.161	.0186	.142	.935	2225	
20	.148	.0189	.129	.935	0100	
10	.132	.0167	.115	.935	0110	
1	.111	.0120	.099	.917	0123	1.1
20	.139	.0151	.124	.929	0405	
10	.133	.0131	.120	.944	0420	
1	.133	.0130	.120	.944	0430	
Averages						
20m	.172	.0186	.153	.934		
10m	.145	.0161	.129	.938		
1m	.138	.0148	.123	.931		
Overall	.152	.01653	.1343	.9347		
Variability	± 35%	± 25%	± 35%	± 1.5%		

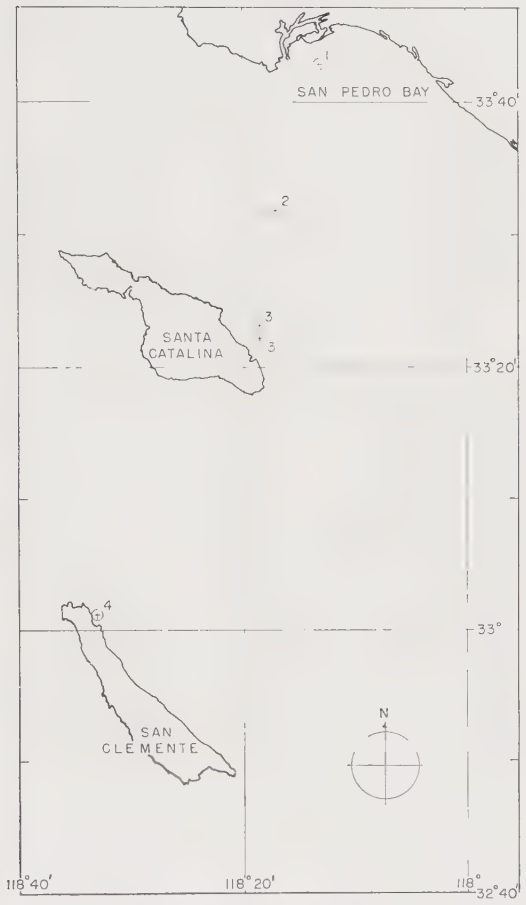

FIG. 1. Chart showing location of coastal and offshore stations where the volume scattering function has been measured.

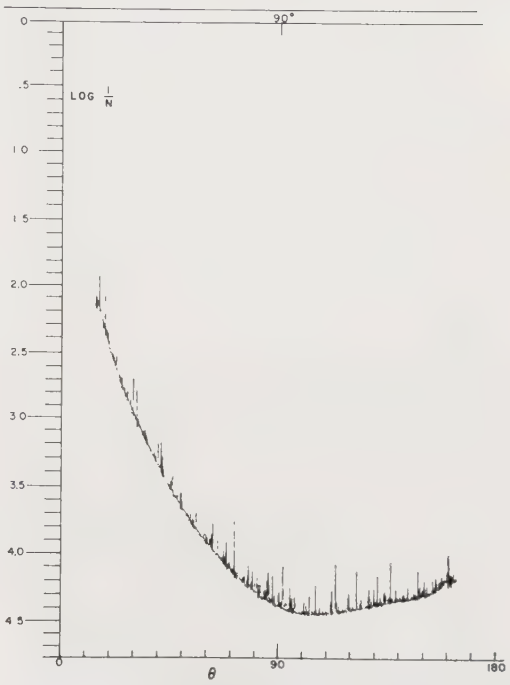

FIG. 2. Data record from a depth of 20 m at Station 4, showing bioluminescent flashing superimposed on the scattering data. This record is uncorrected for changes in the sample volume.

at high light levels, ± 10% at the extreme low light levels.

3. *Volume calibration*—The sample volume has been determined by a method very similar to that described by Pritchard and Elliott (1960). Since the volume is defined by optical and mechanical components, which do not change their characteristics as readily as electrical components, a volume calibration, made near the beginning of these measurements in 1959, was used throughout. The volume calibration consisted of 59 determinations of the sample volume at 15 different angular settings. Maximum uncertainty in the determination of the sample volume is ± 12%.

4. *Irradiance input calibration*—Irradiance input has been determined from measurements on the beam itself using the instrument's own multiplier phototube in order to cancel out optical and circuit gain factors. The calibration procedure has been repeated 5 times during the course of this work and, in between these calibrations, the irradiance input has been controlled by regulation and control of the lamp voltage to ¼%. The accuracy of the irradiance input calibration is estimated to be ± 10%.

The sources of inaccuracy in these measurements do not characteristically vary during short periods of time. Thus, values of the volume scattering function in a single determination can be expected to describe accurately the shape of the curve but there is a high degree of probability that the values reported will collectively be too high, or too low, by 10%. Similarly there is a high degree of probability that data taken during one month will be inconsistent with data taken during another month by ±10%.

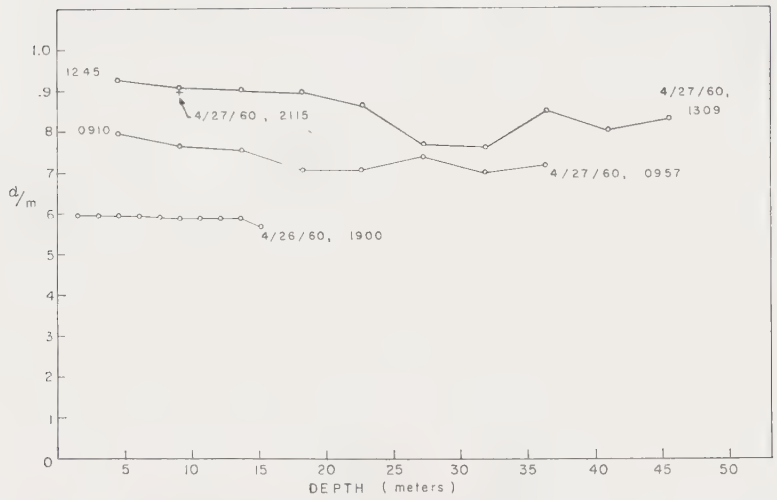

FIG. 3. Showing the variation of α with depth at Lake Pend Oreille on a calm day (April 26, 1960) and at various times during the following day, which was very windy. Bandwidth limited by a Wratten No. 45 filter.

Because of the circuitry employed the lake and ocean data presented here have been obtained at night and the distilled water data has been obtained in a darkened tank.

RESULTS

Table 1 gives the volume scattering function and other optical data for two samples which were purchased as distilled water. Neither sample is regarded as being distilled in the strict sense of the word. The two samples do, however, provide a convenient base for comparison. The filter used has its maximum transmittance at about 522 mμ and has a halfband width of about 80 mμ. Sample B compares favorably

TABLE 4. *Variability in the optical properties of coastal water at Station 4. Data taken in January, 1961. Bandwidth limited by a Wratten No. 57 filter*

Depth (m)	α (per m)	s (per m)	a (per m)	f/s	Time	Tide (ft)
20	.153	.0154	.138	.938	0230	
10	.162	.0149	.147	.935	0245	1.4
1	.159	.0123	.147	.935	0255	
20	.176	.0157	.160	.943	1920	
10	.186	.0163	.170	.945	1935	
1	.170	.0131	.157	.939	1945	
20	.165	.0154	.150	.936	2210	
10	.152	.0141	.138	.943	2230	4.6
1	.139	.0108	.128	.924	2240	
20	.167	.0157	.151	.937	0100	
10	.162	.0166	.145	.935	0110	
1	.162	.0143	.148	.930	0125	
					0850	6.9
					1558	–1.7
Averages						
20m	.165	.0156	.150	.939		
10m	.166	.0155	.150	.940		
1m	.158	.0126	.145	.932		
Overall	.163	.0146	.148	.937		
Variability	± 10%	± 20%	± 11%	± 1.1%		

TABLE 5. *Volume scattering function for Lake Pend Oreille before and after a high wind. Bandwidth limited by a Wratten No. 45 filter*

Sample date	April 26	April 27
Total attentuation coefficient α/m	.589	.909
Total scattering coefficient s/m	.258	.585
Absorption coefficient a/m	.331	.324
Forward scattering coefficient f/m	.248	.559
Backward scattering coefficient b/m	.00976	.0256
Ratio f/s	.960	.955
Volume scattering function $\sigma(\theta)$		
$\theta =$ 0°		
10		
20	.222	.470
30	.0715	.166
40	.0291	.0758
50	.0137	.0380
60	.00712	.0206
70	.00416	.0121
80	.00271	.00780
90	.00198	.00559
100	.00162	.00448
110	.00147	.00394
120	.00143	.00379
130	.00145	.00372
140	.00149	.00371
150	.00156	.00383
160	.00163	.00396
170	.00168	.00406
180	.00170	.00410

with the distilled water described by Hulburt (1945). The ratio of f/s for distilled water is lower than it is for the lake and ocean samples. This would be expected as a consequence of predominately smaller particle size in the cleaner water. The minimum value of the volume scattering function occurs at 100° in both samples.

Table 2 gives the volume scattering function and other data for coastal and off-shore water in the Los Angeles—San Diego area. Station locations are shown in Figure 1. Station 1 is in the outfall area of a sewage disposal plant. The water at Station 1 was full of phosphorescent microorganisms. However, the relative motion between the scattering meter and the water was very slow at this station and phosphorescense was not seen during the measurements. It is unlikely that the relative motion between instrument and water caused enough phosphorescence to affect the measurement.

Stations 2, 3, and 4 were outside the sewage outfall area but were full of flashing bioluminescent organisms. A typical record from Station 4 at 20-m depth is shown in Figure 2. Relative motion between instrument and the water was fairly rapid at Station 4 and the organisms could be seen flashing as they bumped into the various parts of the scattering meter. The same Wratten No. 57 filter was used in these measurements. Note that the angular position of the minimum value of the volume scattering function varies from 100° to 120° in these data.

TABLE 6. *Variability in the optical properties of Lake Pend Oreille as a function of depth before and during a high wind. Bandwidth limited by a Wratten No. 45 filter*

Depth (m)	α (per m)	s (per m)	a (per m)	f/s	Date
4.6	.594	.236	.348	96.4	
9.2	.589	.258	.331	96.6	April 26, 1960
13.8	.589	.245	.344	96.1	
9.2	.909	.585	.324	95.5	
18.2	.897	.559	.338	95.2	April 27, 1960
24.7	.865	.585	.280	95.7	

Tables 3 and 4 show the principle features of the time and depth variability of the water at Station 4 — again using the Wratten No. 57 filter. In Table 3 the water appears to become more absorbing and more scattering with depth and exhibits a high degree of variability. In Table 4, the water is much less variable with time and depth. The ratio of f/s is substantially constant for both days and for all depths and times, indicating that the particle shape is not varying significantly.

Table 5 gives the volume scattering function for lake water at the southern end of Lake Pend Oreille, Idaho, just before (April 26) and after (April 27) a strong north wind. These data were taken with a Wratten No. 45 filter which has a peak transmittance at 480 mμ and a half bandwidth of about 60 mμ. Minimum scattering here occurs at 120° and 140° and the ratio f/s is the highest reported.

There has been an obvious increase in scattering following the high wind. The changes in α are followed in a little more detail in Figure 3 which shows α as a func-

tion of depth on April 26 at 1900 and for two periods during April 28 with one check point taken at 2115. The slopes of the two curves for the 28th suggest that wave action along the shores is introducing scattering material into the surface water which is being carried away from shore by the wind and mixed by wave action. Disturbing of bottom sediments at the station seems unlikely because of the 750 ft of depth.

Table 4 gives additional data on the variability of the lake water with depth on these two days.

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A PORTABLE PHOTOCELL FLUOROMETER FOR DILUTION MEASUREMENTS IN NATURAL WATERS[1, 2]

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ABSTRACT

A portable fluorometer for use with a fluorescent dye tracer in field measurements of dilution rates in natural waters has been designed and tested. The unit is inexpensive, accurate, and reliable. It requires only a small amount of a-c power.

The instrument has been used in a series of 24 measurements of the dilution rate in the surface waters of Little Traverse Bay, Lake Michigan. The dilution of a quantity of dye introduced into the Bay is given by: $D(T) = r_t^{T/t}$ where T is the time in minutes, and t is a quantity expressing the dilution period of interest. The average value for the observed dilution ratio, under winds up to 10–12 mph, is $(r_{10})_{av} = 2$ (*i.e.*, an average dilution of x2 every 10 min).

INTRODUCTION

Many problems of extreme practical interest to the limnologist and the sanitary engineer involve the dilution rates of influent waters and/or of contaminants in lakes and streams. Fluorescent dyes are demonstratedly excellent tracers of water movements, and, with suitable instrumentation, will also yield simultaneous data on the dilution rates inherent in the traced currents. This paper describes, and gives results from, a portable fluorometer developed for use with the fluorescent dye Rhodamine B (Pritchard 1960); the fluorometer is inexpensive, accurate, reliable, and portable.

Other diffusion studies reported in the literature have used fluorescein as the tracer and photomultiplier tubes as the detecting elements. The fluorometer described by Moon, Bretschneider, and Hood (1957) uses a photomultiplier to detect the presence of fluorescein with the same sensitivity of operation as the instrument described in the present paper. The visible-light filters in the photocell fluorometer may be changed to accommodate fluorescein which is somewhat cheaper than the Rhodamine B used in this experiment. A commercial fluorometer which offers two orders of magnitude greater sensitivity is offered by G. K. Turner Associates.

INSTRUMENTATION

In order to maintain a flexibility of operation, the present equipment uses the inter-connection of several components rather than the construction of a single unit containing the fluorometer, sampling pump, and recorder. Figure 1 shows a block diagram of the complete fluorometer system. We have used an electric pump, operated from a portable generator, for continuous sampling. A hand pump for continuous sampling, or a batch process could also be used. The recorder may be either a recording potentiometer or a voltmeter, the requirements being that the measuring device must have a high input impedance and must be capable of covering a wide range of voltages. Using the suggested photocells and battery, an output voltage range of 0.1 to 35 v has been obtained.

Figure 2 shows a schematic diagram of the construction of the fluorometer. The visible light emitted, under ultraviolet radi-

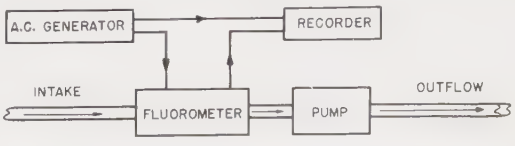

FIG. 1. Block diagram of the complete fluorometer system.

[1] Supported in part by Consumers Power Co., Jackson, Mich.

[2] Contribution No. 10 from the Great Lakes Research Division, Institute of Science and Technology, the University of Michigan, Ann Arbor.

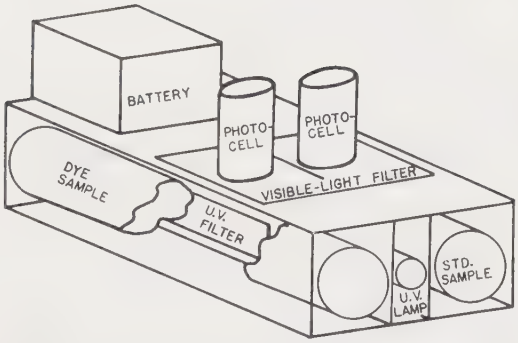

FIG. 2. Schematic diagram of fluorometer construction. In use the instrument stands with the left end down and the intake enters the bottom of the dye-sample tube.

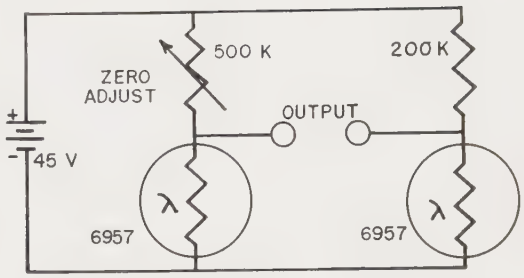

FIG. 3. Schematic diagram of the fluorometer circuit.

ation, by water containing a fluorescent dye is compared with the emission from a standard cell containing natural lake water. A comparative technique is used to obtain reliability of measurement in the possible presence of variations in the intensity of the ultraviolet light source, systematic changes in the characteristics of the photocells, or differences in the amounts of naturally occurring fluorescent substances in the lake water.

A single 4-w ultraviolet lamp (Sylvania F4 T5/BLB) was used to illuminate the water in the sample and standard cells. The ultraviolet was filtered with Wratten No. 18A filters to remove as much visible light as possible. The sample cells were Plexiglas tubes of 1¼-in. internal diameter oriented parallel to the axis of the UV light. Plexiglas was used to obtain maximum transmission of the UV.

The sample tubes were mounted with their axes vertical so that any bubbles in the tubes would rise out of the range of the photocells. The standard cell consisted of a single sample of the regional water which was kept in a stoppered tube, while the dye sample was continuously pumped through a second tube equipped with hose fittings. The intake hose was connected to

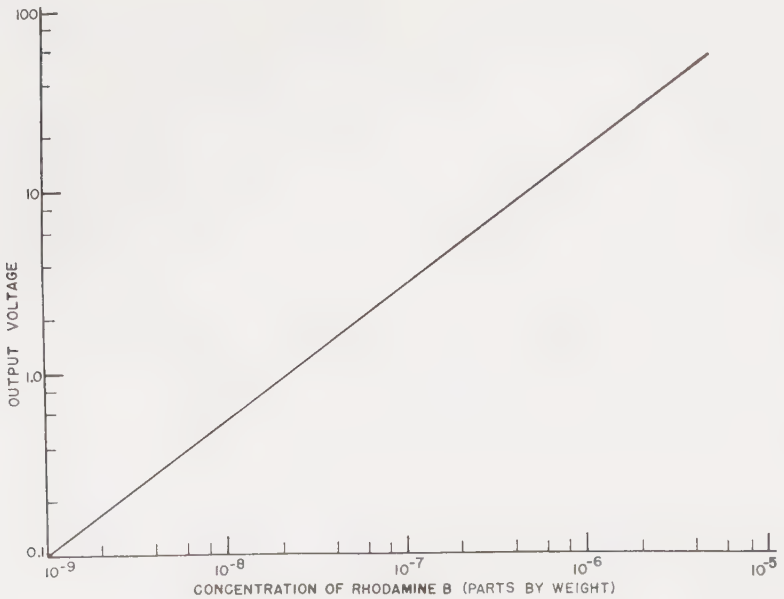

FIG. 4. Fluorometer calibration curve.

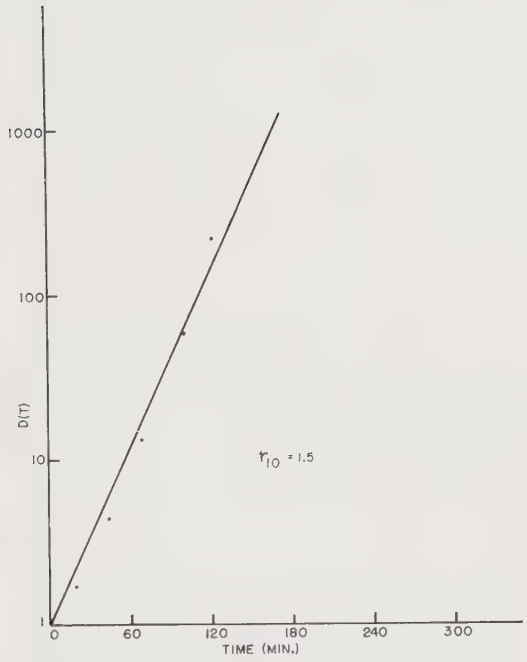

FIG. 5. Representative dilution curve. $D(T) = C_0/C(T)$. Single dye patch.

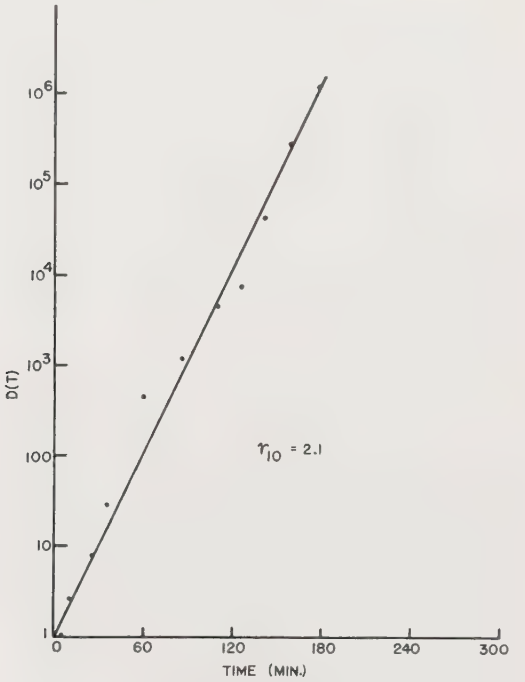

FIG. 6. Representative dilution curve. $D(T) = C_0/C(T)$. Dye patch recharged at $T = 35$ min, and again at $T = 125$ min.

the bottom of the sample tube, and the sampling pump connected to the top, to eliminate any bubbles in the tube. The sampling pump was placed in the outflow from the fluorometer to prevent a possible accumulation of dye in the pump from contaminating the dye sample. The entire fluorometer was placed in a light-tight box to eliminate the effects of ambient light upon the photocells.

The photocells were oriented at 90° to the source of UV light incident upon the sample cells so that direct radiations from the light source could not be received by the photocells. Visible-light filters were placed between the sample cells and the photocells so that the photocells would respond only to the fluorescent wavelength of the dye contained in the sample water. In the present case, Rhodamine B dye was used with a Wratten 25 filter. Rhodamine B was chosen because of its fluorescence in visible light (thus permitting visual observation of the behavior of the dye patch) and because its fluorescent wavelength is not apt to be duplicated by substances naturally present in the water prior to the introduction of the dye.

The schematic diagram of the electrical circuit of the fluorometer is shown in Figure 3, and the calibration curve is shown in Figure 4. The fluorometer was calibrated by using standard-sample tubes containing known concentrations of dye in water taken from the region of interest. The use of the regional water minimizes the possibility of fluorescence of contaminants interfering with the results of the measurements.

By using a vacuum-tube voltmeter to measure the output signal from the fluorometer, the a-c power demands for the voltmeter and fluorometer would be sufficiently small to permit operation with a converter and a storage battery. Thus, if a handpump were used for sampling, the total unit would be sufficiently portable for field studies of the limnology of remote regions.

EXPERIMENTAL METHODS

Since the purpose of the program was the measurement of the dilution rates of

surface waters, the stock dye solution was diluted with methanol in order to obtain a solution of specific gravity such that the dye patch would stay on the surface of the water during the course of the measurements. In this series of measurements, one pint of a stock solution of Rhodamine B in 40% acetic acid solution was mixed with three quarts of methanol. The mixture stayed in the upper 10 ft of the water, with the major portion of the dye less than 3 ft from the surface.

After the dye was introduced, the research vessel was slowly coasted through the dye patch while a continuous sample was being pumped through the fluorometer. Successive passes were made through the dye, and the maximum observed dye concentration was recorded for each pass. By recording the concentration maxima, a minimum rate of dilution was obtained. A typical sequence might consist of passes through the dye patch every 10 or 20 min for a period of 2 hr (until the dye patch is too diffuse to measure accurately). As can be seen from the calibration curve, a dye concentration of 10^{-9} parts by weight in water can be measured quantitatively, and experience has shown that this concentration can be observed visually in the clear waters of Little Traverse Bay.

The path of the dye patch is observed, and if a prolonged sequence of measurement is required, a second dye patch may be introduced at the leading edge of the first patch when the first patch has become too diffuse to measure. The dilution of the second patch is then observed, and the total dilution occurring through the sequence of the two patches is the product of the dilution observed for the first patch multiplied by that of the second patch. A sequence of any number of patches may be treated in this manner.

The dye concentration as a function of time $C(T)$ is taken to be:

$$C(T) = C_0 f(T) , \qquad (1)$$

where $C(T)$ is the maximum concentration observed in the dye patch at time T, and C_0 is the initial concentration, $C_0 = C(T = 0)$.

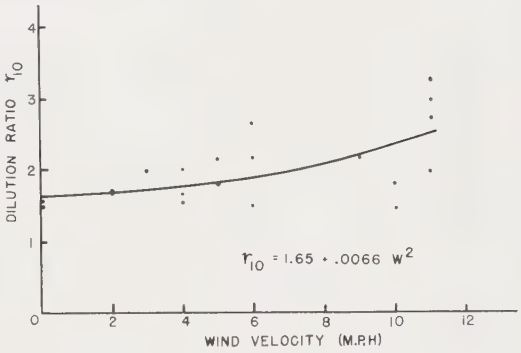

FIG. 7. Dilution ratio as a function of wind speed at the time of measurement.

The dilution is defined as:

$$D(T) = C_0/C(T) = 1/f(T) . \qquad (2)$$

From relation (2), it is seen that the determination of the maximum values of the dye concentration (considered to be $C(T)$) will give minimum values for the dilution $D(T)$. Figures 5 and 6 show representative dilution curves obtained from the sequence of dilution measurements.

From the curves, it is seen that the dilution obeys the relation:

$$\log D(T) = BT = -\log f(T) \qquad (3)$$

where B is interpreted as:

$$B = \frac{1}{t} \log r_t . \qquad (4)$$

The expression for the dilution becomes:

$$D(T) = r_t^{T/t} . \qquad (5)$$

Equation (1) may therefore be written as:

$$C(T) = C_0 r_t^{-T/t} . \qquad (6)$$

In the above expressions, t specifies the dilution period and r_t is the dilution ratio (*i.e.*, r_t denotes a dilution by a factor of r in a period of t minutes).

Consideration of the mechanics of the dilution process indicates the significance of r_t. The total amount of dye in the dye patch is a constant, and if a uniform concentration through the patch is assumed, the product of the dye concentration, C, with the total volume of the dye solution, V, is a constant:

$$CV = B . \qquad (7)$$

TABLE 1. *Frequency distribution of dilution ratios (24 observations)*

Dilution ratio (r_{10})	No.	Dilution ratio (r_{10})	No.
1.4	3	2.1	3
1.5	3	2.6	2
1.6	3	2.9	1
1.7	2	3.0	3
1.9	1	3.2	1
2.0	2		

$(r_{10})_{av} = 2.0$

TABLE 2. *Frequency distribution of current speed (24 observations)*

Current speed (mph)	No.	Current speed (mph)	No.	Current speed (mph)	No.
0.09	1	0.27	2	0.42	1
0.10	1	0.29	1	0.57	1
0.15	1	0.31	1	0.64	1
0.16	1	0.34	1	0.65	1
0.18	1	0.35	1	0.68	1
0.20	1	0.38	1	0.71	1
0.21	2	0.41	1	1.05	1
0.25	1				

Av Current Speed = 0.34 mph

Considering the dilution to proceed at a constant rate, the expression for the volume of the dye patch becomes a geometric series in time and may be written as:

$$V(T) = V_0\, r_t^{(T/t)} \tag{8}$$

where V_0 is the initial volume of the dye patch, and r_t is the dilution ratio. Substituting equation (8) into equation (7), the expression for the dye concentration as a function of time becomes:

$$C(T) = (B/V_0) r_t^{-(T/t)}$$
$$= C_0\, r_t^{-(T/t)} \tag{9}$$

This is the same expression as that obtained from the dilution measurements (equation 6), demonstrating that the dilution may be considered to proceed as a simple geometrical process.

EXPERIMENTAL RESULTS

The frequency distribution for the values of r_t which have been obtained from the data is given in Table 1. The corresponding distribution of observed current speeds for the motion of the dye patches is given in Table 2. The relation of the dilution ratio to the wind speed at the time of measurement is shown in Figure 7. The curve has been fitted to the data by the least squares method (Goedicke 1953). The dilution ratio has been found to be independent of both the current speed and the distance traveled by the dye patch.

The average value of the dilution constant, under light winds, is 2.0 for $t = 10$ min, with a range of 1.4 to 3.2. The average value for the current speed of the dye patch is 0.34 mph, under light winds, with a range of 0.09 to 1.05 mph. The relation between the dilution ratio and the wind speed is given by:

$$r_{10} = 1.65 + 0.0066\, W^2. \tag{9}$$

The experimental method, in this case, has been designed to yield figures for the minimum dilution rate in the surface waters of Little Traverse Bay. In computations of the expected dilution, it is necessary to use the straight-line *transport velocity* of the water mass (as opposed to the current speed of the dye patch along a meandering path) to obtain the time available for dilution as the contaminant moves from its point of origin to the region where its presence is of concern (the region for which the predicted dilution is computed).

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A METHOD FOR SORTING BOTTOM FAUNA SAMPLES BY ELUTRIATION[1]

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ABSTRACT

A sorting device based on mechanical agitation of a substrate sample in a water column, followed by elutriation, provides a rapid and convenient method of separating benthic faunal components and finer sediment fractions. The operation is efficient in terms of total time of analysis and the percentage of animals removed. The method is versatile and can be modifed to meet the needs of specific sampling programs.

INTRODUCTION

During the course of a study on the role of substrate in fauna distribution on stream bottoms, a sorting tube was developed for separating macro-invertebrates from substrate samples. The same device was employed to remove silt and clay fractions from substrate samples in preparation for mechanical analysis (Cummins 1961). The sorting apparatus, or "bubbler," relies on mechanical agitation of a substrate sample by a battery of small air and water jets which place all but the heaviest materials in suspension in a water column. Separation is achieved through differential settling rates resulting from variations in the specific gravity and general shape of suspended materials. Further separation can be obtained by employing a series of small graded sieves below the outflow when the desired suspended fraction is tapped off.

The removal of macro-invertebrates from benthic samples is extremely time consuming and accuracy often varies according to the particular procedure employed. Jonasson (1958) has discussed aspects of sieving with particular regard to mesh size, while Anderson (1959) has reviewed the various flotation techniques for the sorting of macro-invertebrates from sieved substrate samples and described his own method employing a sugar solution. Moon (1935) and Allen (1951) used variations of an elutriation technique for sorting stream samples but their devices were quite cumbersome.

The bubbler described below has proven to be very effective in separating the benthic invertebrate fauna from a variety of lake and stream sediments. It offers considerable savings in time over the more conventional sieving and hand-picking or flotation methods, as well as separating the organisms into general size categories. The device is quite versatile since a whole substrate sample can be treated in the case of a small sampler, or large samples can be proportioned or can receive a preliminary sieving in the field to reduce their volume prior to sorting.

CONSTRUCTION

Figure 1 illustrates the construction of the bubbler; none of the dimensions are critical except the length of the main tube which must be sufficiently long to permit rapid agitation of the sample without overflow. The main tube of the bubbler and the drain spout are made from acrylic plastic tubes. The plunger brace (guide) and the three base pieces are cut from ⅜-in. plexiglass. The plunger brace is glued inside the drain spout with a general purpose acrylic cement; wire brads provide a necessary anchor for this piece. The drain spout and upper base piece (insert 1) are

[1] This apparatus was designed and constructed in connection with a project supported by the National Institutes of Health.

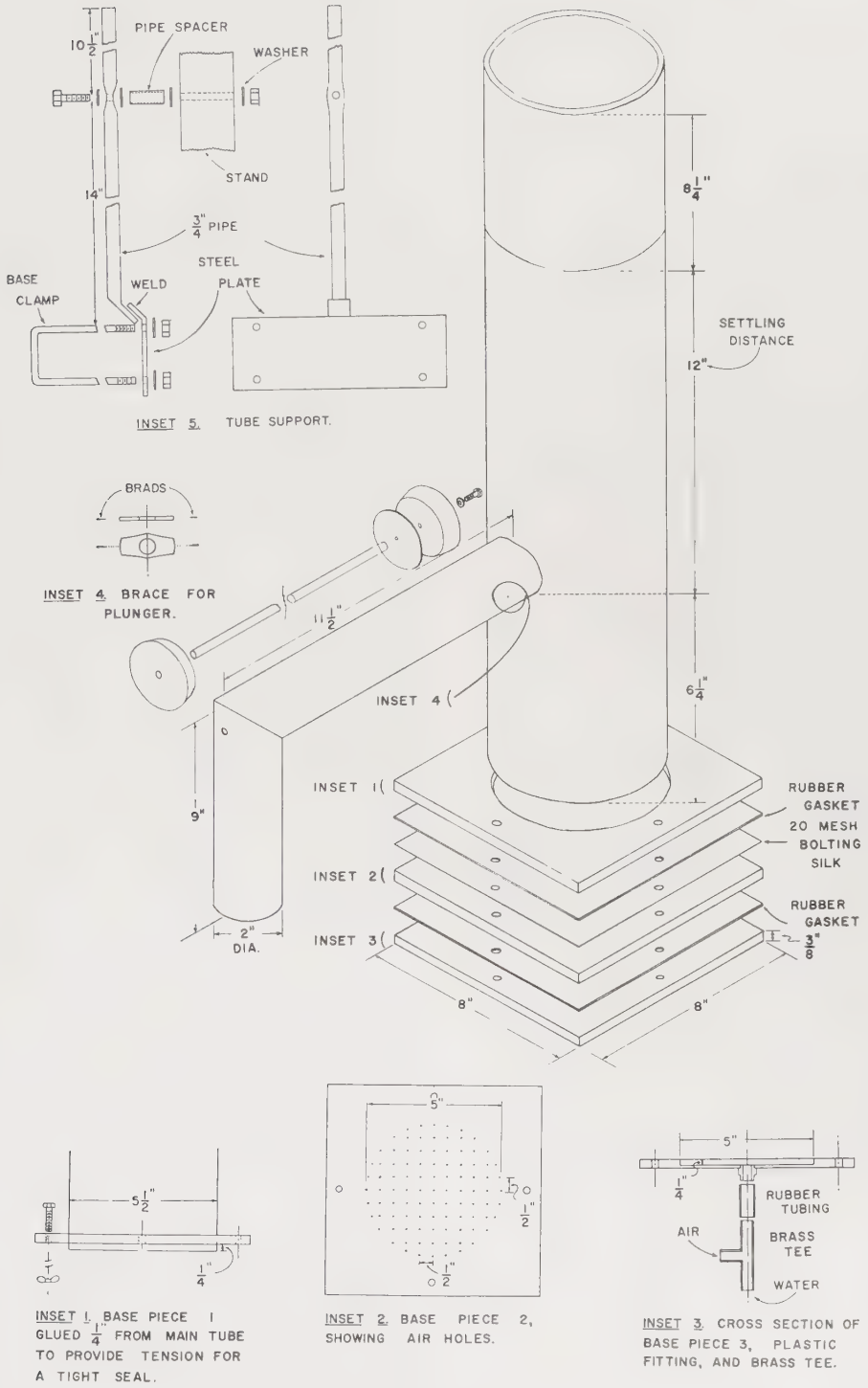

FIG. 1. An apparatus for sorting bottom fauna samples by elutriation.

securely glued to the main tube. The latter is attached approximately ¼ in. from the end of the tube to permit adequate tension to be developed, thus providing a tight seal between the base components. The central piece (insert 2) is perforated by a number of minute holes (1/16 in. diameter), while the lower base piece (insert 3) has a recess ¼ in. deep and 5 in. in diameter to provide a uniform distribution of both air and water. A short, rigid plastic tube facilitates the attachment of the combined air–water inlet. The seal between the base pieces is maintained by rubber gaskets cut to fit. A piece of #20 silk bolting cloth is inserted above the central base piece to prevent clogging of the small holes. Wing nuts used to hold the base together facilitate quick take-down for replacement of the bolting silk.

The plunger consists of rubber stoppers secured to each end of a brass rod. The stopper which closes the outlet inside the main tube is bolted against a brass plate and into the rod. A spring (not shown) between the other stopper and the angle of the drain spout maintains tension on the plunger and prevents leaking.

The bubbler support is a ¾-in. pipe bent and welded to a steel plate, the latter having four holes for two U-bolts which clamp the base pieces to the support. Near the middle and top of the bubbler, rubber tubing is fastened around the main tube and support pipe for additional anchorage. This combination is then attached to the main stand (a stable vertical pipe) by a bolt through the support pipe and stand, with a spacer in between, which allows the apparatus to be pivoted or inverted for cleaning. The stand should be mobile, or installed adjacent to a laboratory sink; it could be modified to be mounted on a sink drain-board.

Both the water and air inlets should be controlled by appropriate faucets or stopcocks.

PROCEDURE

Though there are several possible procedures for using the bubbler, the following was demonstrated to give best results and was employed in obtaining the data presented below. After adding several inches of water to the tube to cushion the fall of heavy substrate materials, the sample was introduced by washing it from the sample container. Additional water was introduced, filling the tube to a line approximately 12 in. above the bottom of the drain spout. It is desirable to remove large cobbles or excessive amounts of vegetation from a sample by washing it through a coarse sieve (20–40 mm mesh) fitted over the top of the tube. Various sizes of sieves can be employed to catch the material tapped from the drain spout. The selection of sieve size is dictated by the size range of animals encountered, the smallest size category to be retained, and the nature of the substrate. The sieves must be inspected carefully since small organisms, especially midges and oligochaetes, tend to catch in drops of water underneath or pass undetected if too large a mesh is employed. We used a sieve with 2-mm mesh above one of 1 mm or 0.5 mm. Compressed air was introduced to agitate the sample sufficiently without loss of material from the top of the tube. From this point we used one of two methods; either the material in suspension was tapped off while the samples were being agitated, or the air was turned off two seconds before pushing the plunger. While the first method suspends most of the heavy animals (such as smaller mollusks and mineral case-bearing caddisflies), the second reduces the substrate caught on the sieves. Depending upon the amount of material retained by the sieves, organisms were picked directly from the sieves or washed into a white tray. Unless there were unusually large amounts of organic detritus, the residue on the screens was examined in a tray only after the fourth and final washing.

When the procedure (filling the tube, agitating the sample, tapping off the suspension, and removing the animals) had been repeated four times, the tube was inverted and the remaining material washed out by opening the water inlet. Four washings appeared to yield an optimum balance between effort and relative sorting effi-

TABLE 1. *Animals recovered in four washings from lake and stream samples*

Samples (location and no.)	Total number recovered	
	Bubbler	Residue
Lake (21)	219	17
Stream (36)	2,420	47

ciency. Occasionally an animal became attached to the bolting silk at the bottom of the tube making it advisable to inspect the tube before the introduction of the next sample.

The effectiveness of the bubbler in sorting benthic organisms was substantiated by determining the animals in the residue after four washings, and by the efficiency of recovery of a known number of naturally occurring benthic invertebrates which were added to specific samples. Twenty-one bottom samples were obtained with a 6-in. Ekman dredge in water depths ranging from 1.5 to 8 m. The substrate was a marly mixture of silt and sand with some vegetation, mostly *Chara*; the percentage of sand decreased with depth. Following conventional practice, the samples were sieved in the field using a screen having a 0.5-mm mesh. Substrate and the organisms retained were preserved and later used in evaluating the performance of the bubbler. Thirty-six bottom samples were taken from a small shallow stream where the substrate was composed largely of gravel, but contained slight amounts of silt, some sand, and pebbles. The samples were obtained using a cylinder sampling device (Cummins 1961), and the total sample was preserved in each case. Both the lake and stream faunas consisted primarily of tendipedid larvae and *Tubifex* worms with some leeches, nematodes, mollusks, amphipods, and various insect groups (Odonata, Ephemeroptera, Megaloptera, Trichoptera, Coleoptera, and other Diptera).

Selected bottom samples from both the lake and stream were treated to remove all animals present. A known number of naturally occurring benthic invertebrates were then added, and the samples were introduced into the bubbler. The known animals were obtained from lake and stream sediment samples which were agitated in the bubbler and washed into a 0.25-mm mesh sieve. A 0.5-mm mesh sieve was selected for the recovery experiments so that the animals retained would be within the size range normally selected by field sieving and hand-picking techniques.

RESULTS AND DISCUSSION

Table 1 gives the number of animals recovered in four washings in the 21 lake and 36 stream samples. The majority of the animals were recovered by the third washing, with the final washing adding only 2% and 4%, respectively. If the number of animals still remaining in the residue is used to determine the effectiveness in removal of the organisms, an average of 92% was recovered from the lake samples while 98% recovery was obtained from the stream samples. Two lake samples contained 12 of the total of 17 animals recovered from the residue, or approximately 70%. The specific reason for the discrepancy is not known, but it is suspected that the routine analysis procedure may have been altered inadvertently. The animals that remained in the residue were primarily midge larvae and *Tubifex* worms. However, their numbers in the residue were approximately proportional to their abun-

TABLE 2. *Recovery in four washings of known numbers of animals introduced into substrate samples*

Samples (location and no.)	Treatment	No. Introduced	Recovery		Residue		Unrecovered	
			No.	%	No.	%	No.	%
Lake (5)	Agitated	104	90	86.5	1	1.0	13	12.5
	Settled	98	90	91.8	5	5.1	3	3.1
Stream (4)	Agitated	355	317	89.3	3	0.8	35	9.9
	Settled	321	282	87.9	15	4.7	24	7.4

dance in the total sample. The bubbler was effective in sorting the smaller mollusks (*Sphaerium*, *Pisidium*, and *Ferrissia*) present; no mollusks were found in the lake sample residue, and only 3% of the total number of mollusks from the stream samples were present in the residue.

The introduction of a known number of benthic animals into substrate samples from which all animals had been previously removed provided an opportunity to determine the efficiency of the bubbler. Both the lake and stream samples indicated a recovery of approximately 89% (Table 2) using either the agitation or settling method. The agitation method was superior in recovering the heavier animals. It did not alter the recovery of the lighter animals, however.

A recovery of 89% of introduced animals is very good, particularly when one considers that a 0.25-mm sieve was employed in obtaining the animals initially. Since a 0.5-mm sieve was used in the comparative recovery trials, the data suggest that 8 to 9% of the animals were lost owing to their being too small to be retained by the larger sieve. The agitation method apparently contributes to the loss of the very small organisms because of the increased velocity of discharge at the drain spout. These animals could usually be recovered in the wash water indicating that the bubbler was actually effective in removing them. With this consideration, the efficiency of the apparatus with known samples can be considered to be approximately 97%.

The length of time required to analyze a sample varied from 15 to 45 min, depending upon the nature of the substrate. An average value for the 57 samples analyzed was approximately 25 min. These times compare favorably with the results obtained by Anderson (1959) using the flotation technique, although close comparison is not possible because he gave no information on sample size or sediment type.

While percentage recoveries obtained with the bubbler equal or exceed those obtained by other techinques, the time required per sample and the convenience of operation also commend the method. The bubbler is especially valuable if used on samples which are to be analyzed in their entirety for substrate particle size. Such samples cannot be partially sieved in the field and hand-sorting is especially laborious. Also, flotation substances which interfere with sediment analysis cannot be employed. In addition, the device can be utilized to separate sediment fractions once empirical settling times have been derived.

The method can be modified to fit the particular needs of a given sampling program. For example, various settling times can be employed depending upon the abundance of different animal groups and the mesh sizes of the catch screens can be chosen so that animals of a desired size are retained.

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MIGRATION AND REPRODUCTION OF THE WOOD-BORING ISOPOD, *LIMNORIA*, IN SOUTHAMPTON WATER

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ABSTRACT

Three species of *Limnoria* occur together in Southampton Water. Migration began at a water temperature rising through 10°C in *L. quadripunctata* and *L. lignorum* and through 15°C in *L. tripunctata*. Migratory activity followed the water temperature in *L. tripunctata* but declined in *L. lignorum* before the highest temperatures were reached. The migratory behavior of *L. quadripunctata* resembled that of *L. tripunctata* at one site and that of *L. lignorum* at another. Overcrowding as a cause of migration was not supported. Migration was confined to large, sexually mature adults, excluding gravid females. The first migrants were predominantly male, and in *L. lignorum* about twice as many males as females migrated.

Reproduction showed a marked increase in the summer, beginning at a water temperature of 10°C in *L. quadripunctata* and *L. lignorum* and of 12°C in *L. tripunctata*. *L. quadripunctata* differed from other species in being the only one in which the average brood size of post-migrants was significantly greater than that of non-migrants, and in which a significant increase in brood size with body size occurred. No seasonal change in brood size was demonstrated in any species. Widely separated developmental stages were frequently found in the same brood. The duration of the egg stage was longer than that of the embryo which in turn exceeded that of the larval stage.

INTRODUCTION

Three species of *Limnoria*, which do not appear to interbreed, occur together in Southampton Water, Hampshire, England (Eltringham and Hockley 1958). The species present are *L. lignorum* (Rathké), *L. quadripunctata* Holthuis and *L. tripunctata* Menzies. Although *Limnoria* has always been a serious economic pest because of its wood-boring activities, surprisingly little is known of its biology. Some work has been done on its reproductive cycle, Sømme (1940), Johnson (1935), Coker (1923), Johnson and Menzies (1956), and in general, it appears that a season of increased reproduction occurs. Most of these investigations have been made on *L. lignorum* but Johnson and Menzies (1956), who worked on *L. tripunctata*, claimed to have found a specific difference in the water temperature at which increased reproduction commences. Data on such reproductive phenomena as the brood size and composition and egg development are very sparse. Henderson (1924), Johnson (1935) and Sømme (1940) gave averages for egg numbers in *L. lignorum* varying from 10 to 35 but the numbers of females examined have been small. However, the average brood sizes found for this species have been higher than that given by Menzies (1954) for *L. tripunctata* (mean brood size 4.7). The only figures available for *L. quadripunctata* are those of Kofoid and Miller (1927) who found a mean brood size of 9.5 in 21 gravid females. Coker (1923) reported a greater number of eggs (maximum 12) per female in the spring than in the summer or autumn broods (maximum 9). His material was probably *L. tripunctata* according to Menzies (1954). An important phenomenon was noted in *L. tripunctata* by Menzies (1954) who found a higher average brood size in gravid females from test blocks than in females from more densely populated piling samples.

The dispersal phase in the life history of *Limnoria* is usually referred to as migration in the literature, but the use of this term does not imply a massive movement of the entire population as in true migration. The published accounts of the migratory behavior of *L. lignorum* indicate that migra-

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tion is confined to a few months in the spring when the water temperature is rising, Kramp (1927), Johnson and Miller (1935), Johnson (1935), Sømme (1940), Black and Elsey (1948), Johnson and Menzies (1956). The migratory activity of *L. tripunctata* was found by Johnson and Menzies (1956) to lag slightly behind the seasonal trend in temperature. No data on the migration of *L. quadripunctata* have been published.

It appears, therefore, that specific differences occur in several of the phenomena noted, but as many of the observations have been made in widely separated localities, the observed differences may have been geographic as much as specific. The occurrence together of the three species in Southampton Water permits a more critical examination of the problem.

We are grateful to Mr. J. P. M. Pannell of the Southampton Harbour Board for permission to take samples in Southampton Water and for the provision of many facilities. The work was carried out during the tenure of a maintenance grant from the Department of Scientific and Industrial Research.

MATERIAL AND METHODS

Southampton Water is a landlocked arm of the sea about 10 miles long with an average width of about 1½ miles. It opens into the Solent, which is a narow channel between the south coast of England and the Isle of Wight. The results were obtained from analyses of test blocks exposed in the sea at three sites in Southampton Water. These were: Site A—Southampton, Royal Pier (50°53′44″ N, 1°24′23″ W), Site B—Marchwood, Husbands Jetty (50°53′47″ N, 1°25′24″ W) and Site C—Calshot Castle (50°49′14″ N, 1°18′10″ W). Piling samples have been examined from Sites A and C.

Site A.—The test blocks at Southampton were exposed primarily to investigate the migratory behavior, but *Limnoria* from these blocks have also been used in reproductive studies. The blocks were of deal, about 30 × 10 × 2.5 cm in size and were exposed at the site about 0.5 m above the mud line and about 1 m above Port Low Water Datum. A block was loosely secured to the rough side of a concrete pile by means of a length of galvanized wire passed around the pile and through a hole bored in the 10-cm axis of the block. A pair of blocks was exposed each month. One of these was withdrawn at the end of a month while the other was exposed for a period of two months. These blocks are referred to below as one- or two-month blocks according to the period of exposure.

Site B.—Battens were exposed at this site as part of a general survey of marine borers in Southampton Water. They were of Corsican pine measuring 150 × 5 × 2.5 cm and were bolted vertically to an iron frame which was suspended from a jetty with its top about 20 cm above P. L. W. D. The battens were not in contact with any part of the jetty and could be reached only by swimming *Limnoria*. One batten was put out each month. After a month's exposure, each batten was superficially cleaned to remove settled barnacles, *etc.*, and then left out for a further month before withdrawal. After this site had been selected for the study, the wooden jetty, which had been extensively damaged by *Limnoria*, was removed and replaced by a metal and concrete structure. Consequently the natural *Limnoria* population was very much reduced and the attack on test blocks was less than that at the other sites.

Site C.—At this site both battens and test blocks were exposed. The battens and their method of exposure were similar to those described above and were used to follow the migration of *Limnoria*, while the test blocks were used for the principal investigation into the reproductive behavior of *Limnoria*. These blocks, measuring 10 × 10 × 20 cm were of pine with planed surfaces. The periods of exposure varied for reasons unconnected with this work; at the beginning the blocks had been in the sea from 18 to 24 months and contained a well established, but not crowded, population of *Limnoria*. Later blocks had been exposed for only 7 months and, when these included the winter period, were more lightly attacked.

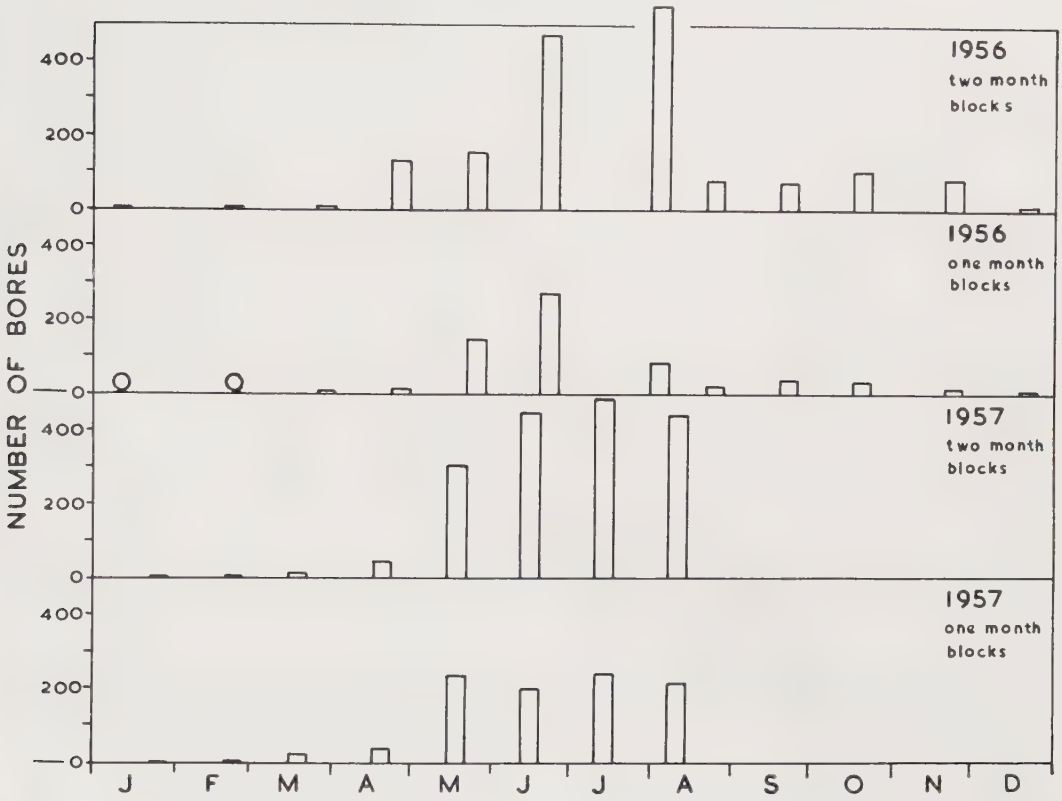

FIG. 1. The migration of the *Limnoria* population at Southampton (Site A). O – signifies the absence of bores from the block.

Continuous temperature recorders were in operation at Sites B and C, except for a period of 3 months from April 21 to July 17, 1956, at Site B owing to a breakdown of the apparatus. During this period daily temperature measurements were made near the site.

Migration

In all cases, the intensity of the *Limnoria* attack was assessed by a count of the total number of bores on the surface of the wood. In 1957, the migratory seasons of the separate species of *Limnoria* were assessed from the number of specimens and bores of each species in a sample of the test block population. The number of bores produced by each species in the whole test block was deduced from the proportion of the species present in the bores of the sample. This number, multiplied by the number of animals per bore in the sample, gave the total number of each species in the test block.

Reproduction

The reproductive level has been expressed as the percentage of gravid females per month in the total adult population, referred to here as the "gravidity." This term was applied by Johnson and Menzies (1956) to the percentage of gravid females in the adult female population, assuming a 1:1 sex ratio. In small samples this ratio may not obtain, and on one occasion these authors found an apparent gravidity of more than 100%. To avoid such confusion, it is better to restrict the term gravidity to the meaning described above.

For work on migrant populations, the gravidity as so defined is an inaccurate index of the reproductive state because the sex ratio of migrant *Limnoria* is neither

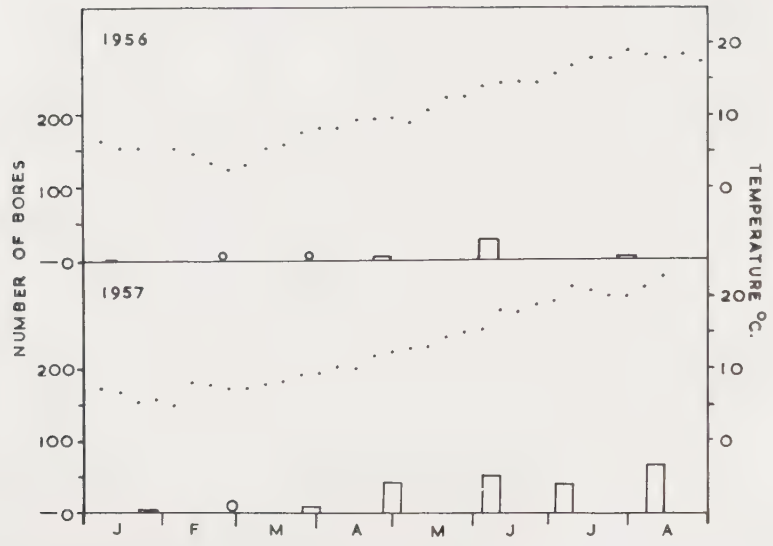

FIG. 2. *Limnoria* attack on battens exposed for two-month periods at Marchwood (Site B). The weekly average water temperatures are included. O – indicates that no bores were found in the batten.

1:1, nor constant. Consequently, our results are expressed as the percentage of gravid females in the total number of females counted in the sample.

Results are given for the *Limnoria* population as a whole, but for the period July 1956–August 1957 the reproductive behavior of each species in the Calshot test blocks has been separated. For this purpose, the specific composition of a subsample of non-gravid specimens was noted, and from this "species spectrum" the number of each species in the total non-gravid population was deduced. The specific composition of the gravid females was calculated from a similar species spectrum. These figures enabled the gravidity of each species to be estimated.

Other reproductive phenomena that have been investigated are the brood size and composition, the variation of brood size with body size in females from test block and piling populations and the sexual composition of migrant populations.

The sex of specimens was determined by the presence or absence of the oöstegites. In all cases in which size measurements were made, *Limnoria* were anaesthetized with a 0.75% solution of urethane in sea water. The width of the pleotelson at its widest point was used as the index of body size. This parameter is more reliable than the length of the animal itself owing to the contraction or expansion of the segments. Use of the pleotelson width as the criterion of size in *L. tripunctata* was made by Johnson and Menzies (1956) who included a table comparing that measurement with the body length.

RESULTS

Migration of the Limnoria *population*

The total number of bores in the battens and test blocks are expressed graphically in Figures 1–3 which include the seasonal water temperature variations. The number of bores in each block is entered in the figure at a point corresponding to the date on which it was withdrawn from the sea. The value, therefore, represents the migratory activity over the preceding one or two-month period. There were very few bores in the test blocks exposed in October 1955 and data from these blocks have not been included in the figure. Some of the Marchwood battens were exposed for periods greater than two months during the winter of 1956 and data from these have also been excluded. Full details of the dates of ex-

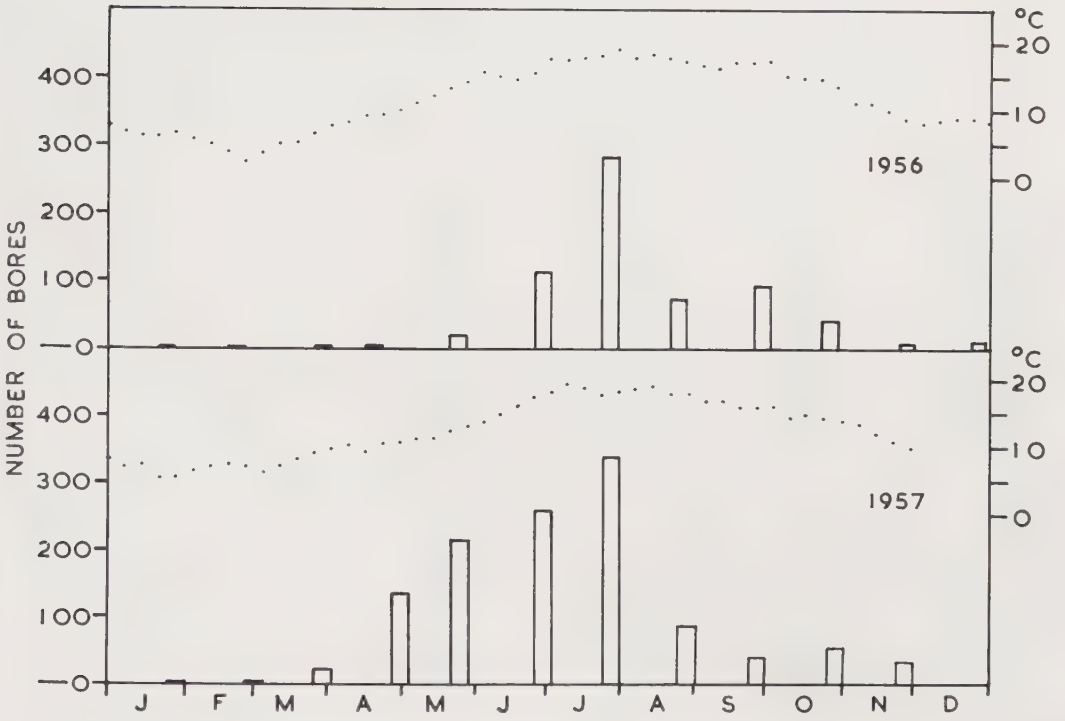

FIG. 3. *Limnoria* attack on battens exposed for two-month periods at Calshot (Site C). The weekly average water temperatures are included.

posure and withdrawal of the test blocks are given in Eltringham (1957).

These results show that at all stations the migratory activity of *Limnoria* was confined to a definite season in the year, with little or no migration taking place during the late autumn or winter months. The migratory season commenced in the spring, reached a peak in early summer and had begun to decline before the maximum water temperatures were reached. This last phenomenon was obvious at all sites in 1956, although to some extent the early decline in migration was masked on the two-month blocks and battens as a result of much of the activity taking place in the first half of the two-month period. A similar pattern was found at Calshot in 1957 but at the two other sites the migratory activity appeared to remain at the maximum level for a longer period than in 1956 and persisted into the warmest months.

Migratory Behavior of the Three Limnoria *Species*

The results of the analyses of one- and two-month blocks from Southampton and of the battens from Calshot are given in Table 1. These analyses have been made on samples taken from test blocks exposed after November 1956 at Southampton and from the first batten at Calshot to show an appreciable level of attack.

Southampton: The calculated total numbers of specimens of each species per block from Southampton are presented in Figure 4 for the migratory period April–August. The migratory behavior was not identical in all species. *L. lignorum* migrated first and, from the evidence of the one-month blocks, appeared to reach its peak migration in May. The maximum migration of the other two species occurred in July, although there was only a slight decline in August. The principal difference between the migratory behavior of these two species

TABLE 1. *Migration of the separate species of* Limnoria. *The number of each species in the test block or batten was calculated from the specific composition of a sample of bores from each block*

Date withdrawn	Number of bores in sample			Total no. of bores in block	Calculated number of bores per species			Number of animals per bore in sample			Calculated number of animals in block		
	trip.	*quad.*	*lign.*		*trip.*	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*
Southampton—one-month blocks													
21.12.56	–	–	4	4	–	–	4	–	–	1	–	–	4
21. 1.57	–	1	–	1	–	1	–	–	1	–	–	1	–
19. 2.57	–	–	2	3	–	–	3	–	–	1.5	–	–	4
19. 3.57	–	4	14	22	–	5	17	–	1	1	–	5	17
18. 4.57	1	14	11	37	1	20	16	1	1.3	1.2	1	26	19
17. 5.57	2	34	125	237	2	50	185	1	1.3	1.3	2	65	240
17. 6.57	13	36	53	199	26	70	103	1.5	1.7	1.6	39	119	165
17. 7.57	21	88	25	241	37	159	45	1.8	1.5	1.8	67	238	81
16. 8.57	12	93	33	214	19	144	51	1.8	1.6	1.7	34	230	87
Southampton—two-month blocks													
21. 1.57	–	1	1	2	–	1	1	–	1	1	–	1	1
19. 2.57	–	2	3	5	–	2	3	–	1	1	–	2	3
19. 3.57	–	1	11	16	–	1	15	–	1	1.2	–	1	18
18. 4.57	–	10	13	50	–	22	28	–	1.3	1.8	–	29	50
17. 5.57	3	20	95	306	8	52	246	1	1.3	1.4	8	68	344
17. 6.57	13	11	62	452	68	59	325	1.6	1.7	1.8	109	100	585
17. 7.57	25	65	39	490	96	247	147	1.9	1.9	1.9	182	469	279
16. 8.57	20	51	29	442	89	225	128	1.9	1.9	1.9	169	427	243
Calshot—battens exposed for two months													
1. 5.57	3	58	28	134	5	87	42	1	1.5	1.6	5	130	67
28. 5.57	18	55	6	213	49	148	16	1.9	1.8	1.8	93	266	29
27. 6.57	67	32	3	255	167	80	8	1.7	1.6	1.7	284	128	14

at Southampton lay in the time of the onset of migration. Migration in *L. quadripunctata* began at about the same time as in *L. lignorum*, but the first appearance of *L. tripunctata* in any numbers on the test blocks was not until two months later in June. Allowing for the fact that the recorded migration took place in the preceding one- or two-month period, it may be concluded that migration in *L. lignorum* and *L. quadripunctata* began in late March or early April while in *L. tripunctata* the onset of migration was delayed until May or early June. *L. lignorum* was the only species at Southampton to show a peak migration on a rising temperature.

Calshot: The migratory behavior of *L. lignorum* and *L. tripunctata* at Calshot was similar to that at Southampton, but a difference was found in *L. quadripunctata* in that a decline in the migration was apparent before the warmest water temperatures were reached. In this respect its behavior resembled that of *L. lignorum*.

REPRODUCTION

Reproduction of the Limnoria *Population*

The results of approximately monthly analyses of the reproductive state in *Limnoria* samples taken from test blocks at Calshot are given in Table 2 and the gravidity is shown diagrammatically in Figure 5. This figure includes the temperature data as the average weekly water temperatures. It is apparent that there was a season of increased reproduction each year. The gravidity was not as high in 1955 as in subsequent years, but the maximum reproduction may have occurred in July when a sample could not be taken. The gravidities shown for the first three months were slightly lower than their true values for, during these months, the immature forms were included in the analyses. A lower level of reproduction continued throughout the year, although it is possible that gravid females found in the coldest months were carrying their eggs through

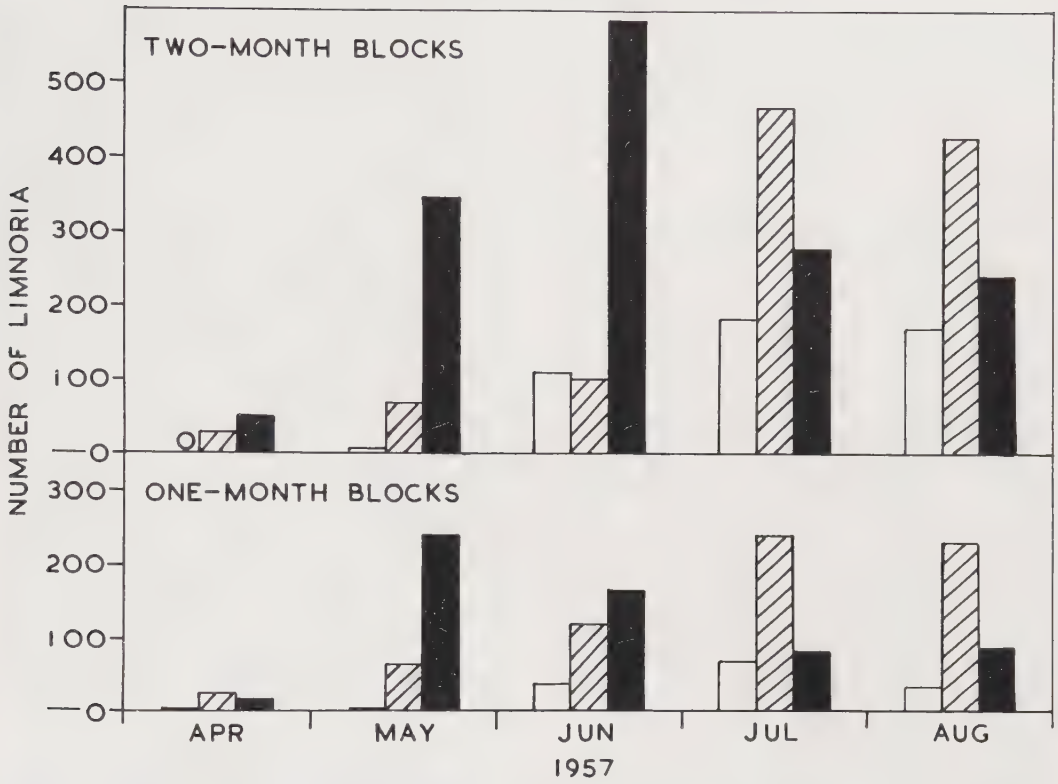

FIG. 4. The migration of the three species of *Limnoria* at Southampton (Site A) for the months April to August 1957. The data are from blocks of one and two months' exposure. Unfilled columns—*L. tripunctata*; hatched columns—*L. quadripunctata*; filled columns—*L. lignorum*. O signifies the absence of bores of *L. tripunctata* from the April sample.

the winter in a dormant state. The reproductive season of 1957 commenced earlier than in previous years. The March figure is not significant, being based on a sample of 5 gravid females in a total of 27 individuals, but the gravidity in May was much higher than it was in the previous May.

A similar examination of samples from infested piling showed that well marked reproductive seasons were also found in these populations.

Reproductive Behavior of the Three Limnoria *Species*

Analysis of the samples of the non-migrant population in Calshot test blocks is given in Table 3, and the calculated gravidity figures per month for each species are shown in Figure 6.

L. tripunctata: The seasonal differences in reproduction were most marked in this species. No gravid females were found in samples collected during the 6 months from November 1956 to April 1957, but during March and April few individuals of this species were taken and the absence of reproduction during these months may not, therefore, be significant.

L. quadripunctata: The decline in the reproduction of *L. quadripunctata* from its maximum in July 1956 was prolonged and extended into December. Few or no gravid females were found between January and March 1957, but increased reproduction had commenced in the sample examined at the beginning of May.

L. lignorum: No specimens of *L. lignorum* were found in the July 1956 sample, but it may be expected that the reproductive state was high. The results of the

TABLE 2. *The reproduction of the* Limnoria *population at Calshot (Site C). Samples taken from test blocks exposed for periods varying from 7 months to two years. The gravidity is the percentage of gravid females in the total adult population*

Date	Total *Limnoria* examined	Total non-gravid adults	Total juveniles	Total gravid females	Gravidity
18.5.55	482			39	8.1
20.6.55	462			104	22.5
11.8.55	521			151	28.9
13.9.55	580	377	108	95	20.1
17.10.55	738	577	95	66	10.3
16.11.55	950	814	71	65	7.4
21.12.55	671	598	24	49	7.6
26.1.56	413	336	19	58	14.7
24.2.56	942	902	16	24	2.6
28.3.56	729	649	33	47	6.7
23.5.56	883	749	44	90	10.7
29.6.56	654	382	23	249	39.5
31.7.56	1,024	530	114	380	41.8
28.8.56	954	588	246	120	16.9
1.10.56	1,052	840	114	98	10.4
27.10.56	1,383	1,079	156	148	13.7
27.11.56	427	336	39	52	13.4
29.12.56	568	448	42	78	14.8
28.1.57	135	104	25	6	5.4
27.2.57	188	131	48	9	6.4
26.3.57	27	22	0	5	18.5
1.5.57	157	113	0	44	28.0
28.5.57	119	97	0	22	18.5
4.7.57	309	193	11	105	35.2

autumn examination were anomalous as samples containing about 20% gravid females alternated with samples containing few or none. There is little certainty about the reproduction of this species in winter (January–March). Although the observed gravidities were high, they were based on inadequate data as very few females of any species were found. In particular, the high gravidity recorded in February is invalid as it is based on 5 gravid females in a total of only 8 specimens. It is probable that these females were carrying young in a dormant condition as suggested above. These results suggest that reproduction in *L. lignorum* was less seasonal than that in the other species, although there appears to have been an increase in reproduction by the beginning of May.

The gravidity in *L. lignorum* was higher than that in *L. quadripunctata* in the early May sample and hence it is probable that the order in which the species showed increased reproduction was: *L. lignorum*, *L. quadripunctata*, and *L. tripunctata*. The period of maximum reproduction was possibly the same in all species, although that of *L. lignorum* was not detected.

The reproduction of post-migrant populations was analyzed in the three species from the test blocks exposed at Site A and the results are shown in Table 4. In addition to the reproductive state, the association of the sexes prior to reproduction was studied each month by noting the number of burrows that were occupied by a pair of

TABLE 3. *The reproduction of the separate species of* Limnoria *at Calshot (Site C). The calculated totals for the individual species are derived from the observed composition of samples taken from the test blocks. The total number of gravid and non-gravid* Limnoria *of all species are given in Table 2*

Date	GRAVID FEMALES						NON-GRAVID ADULTS						Calculated gravidity %		
	In sample			Calculated in test block			In sample			Calculated in test block					
	trip.	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*	*trip.*	*quad.*	*lign.*
31. 7.56	18	42	0	114	266	0	54	172	0	127	403	0	47.3	39.8	–
28. 8.56	5	15	2	27	82	11	135	75	14	355	197	36	7.1	29.4	23.4
1.10.56	8	45	0	15	83	0	45	147	26	174	566	100	7.9	12.8	0
27.10.56	2	21	28	6	61	81	62	42	42	459	310	310	1.3	16.4	20.7
27.11.56	0	22	3	0	46	6	25	52	55	64	132	140	0	25.8	4.1
29.12.56	0	29	14	0	53	25	82	150	53	129	236	83	0	18.3	23.1
28. 1.57	0	0	6	0	0	6	26	8	61	28	9	67	0	0	8.2
27. 2.57	0	3	4	0	4	5	78	38	3	86	42	3	0	8.7	62.5
26. 3.57	0	0	3	0	0	5	1	3	15	1	4	17	0	0	22.7
1. 5.57	0	10	32	0	10	34	2	38	48	3	49	61	0	16.9	35.8
28. 5.57	3	11	8	3	11	8	19	48	30	19	48	30	13.6	18.6	21.0
4. 7.57	45	51	9	45	51	9	91	71	17	98	77	18	31.5	39.8	33.3

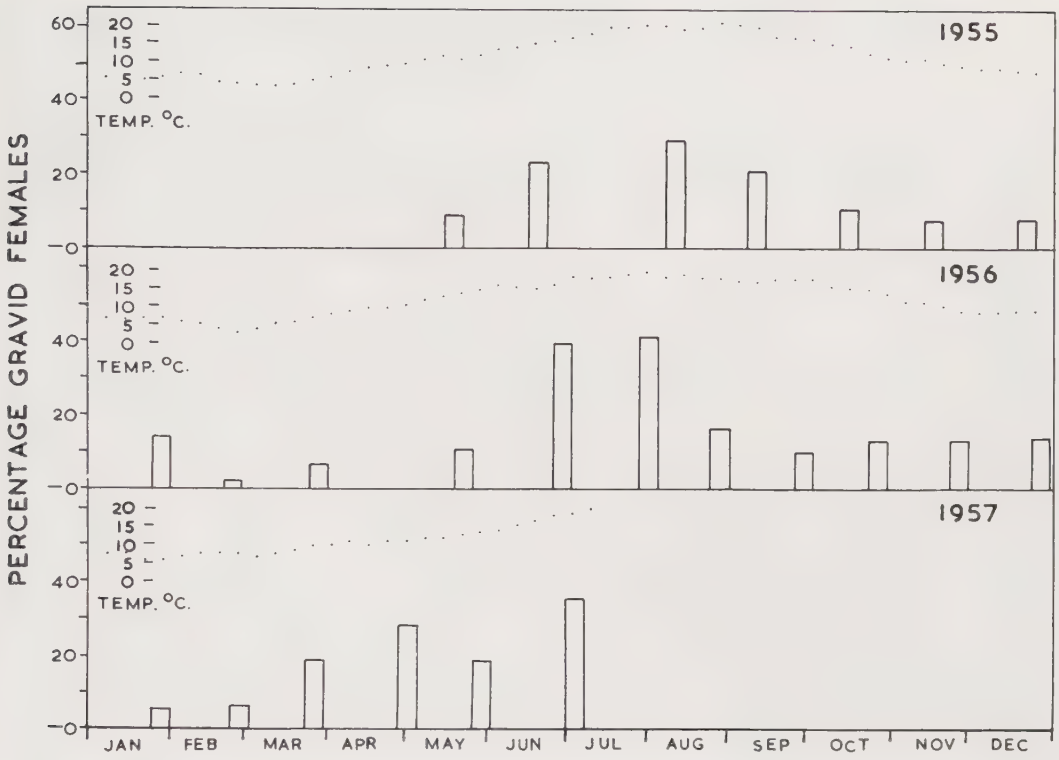

FIG. 5. The reproduction of the *Limnoria* population in the test blocks at Calshot (Site C) from May 1955 to July 1957. The weekly average water temperatures are included.

animals of opposite sex. The results have been expressed as the percentage of those burrows in the total number of burrows. This figure is an index of pairing.

The percentages for the 4 months from April to July 1957, when reproductive activity was in evidence, are shown diagrammatically in Figure 7. The April samples did not contain any gravid females, but in both the one-month and the two-month blocks many of the first migrants had associated in pairs and were probably responsible for the reproduction in May. Reproduction lagged behind pairing by about one month. The reproductive level of *L. lignorum* was low in the one-month blocks although in the corresponding two-month blocks high percentages of gravid females were found. This difference was less marked in the other species and suggests that sexual development took longer in *L. lignorum* and that one month was not sufficient for the full reproductive potential to be manifested.

In the two-month block withdrawn in July, the high proportion (about 90%) of burrows of all species containing a pair of animals suggests that migration was soon followed by reproduction. This is borne out by the very high percentage of gravid females found in this test block and by the order in which the species showed increased reproduction. This order was the same as that in which the species migrated, namely *L. lignorum* and *L. quadripunctata* followed by *L. tripunctata*. A similar situation was found at Site C by an analysis of three test battens.

Brood Size in Gravid Females

Table 5 shows the results of the measurements made to investigate Menzies' observations (1954) that the mean brood size is smaller in gravid females from piling than in those from less densely populated

TABLE 4. *Pairing and reproduction in samples of migrant* Limnoria *taken from test blocks exposed at Southampton. The percentage reproduction refers to the percentage of gravid females in the total number of females present*

Date withdrawn	PAIRING									REPRODUCTION								
	L. tripunctata			*L. quadripunctata*			*L. lignorum*			*L. tripunctata*			*L. quadripunctata*			*L. lignorum*		
	S.[1]	P.[2]	%	S.[1]	P.[2]	%	S.[1]	P.[2]	%	No.	Gravid	%	No.	Gravid	%	No.	Gravid	%
One-month blocks																		
21.1.57	0	0	–	1	0	–	0	0	–	0	0	–	0	0	–	0	0	–
19.2.57	0	0	–	0	0	–	1	1	50.0	0	0	–	0	0	–	1	0	–
19.3.57	0	0	–	4	0	–	14	0	–	0	0	–	1	0	–	3	0	–
18.4.57	1	0	–	10	4	28.6	9	2	18.2	0	0	–	11	0	–	2	0	–
17.5.57	2	0	–	25	9	26.5	88	37	29.6	0	0	–	23	1	4.3	45	0	–
17.6.57	7	6	46.1	9	27	75.0	20	33	62.3	9	1	11.1	31	12	38.7	33	2	6.1
17.7.57	5	16	76.2	44	44	50.0	6	19	76.0	17	5	29.4	49	22	44.9	21	4	19. 0
Two-month blocks																		
21.1.57	0	0	–	1	0	–	1	0	–	0	0	–	0	0	–	0	0	–
19.2.57	0	0	–	2	0	–	3	0	–	0	0	–	0	0	–	0	0	–
19.3.57	0	0	–	1	0	–	9	2	18.2	0	0	–	0	0	–	2	0	–
18.4.57	0	0	–	7	3	30.0	1 2	11	84.6	0	0	–	8	0	–	11	0	–
17.5.57	3	0	–	13	7	35.0	61	34	35.8	2	0	–	13	2	15.4	36	1	2.8
17.6.57	5	8	61.5	3	8	72.7	9	53	85.5	10	2	20.0	8	2	25.0	54	30	55.5
17.7.57	3	22	88.0	5	56	91.8	5	34	87.2	29	23	79.3	66	43	65.1	34	28	82.3

[1] S = burrow occupied by one animal.

[2] P = burrow occupied by a pair.

test blocks. Measurements made on mixed species supported this contention, but when the individual species were examined, a clear distinction was found only in *L. quadripunctata*. In *L. tripunctata*, the brood size was of the same order in both test blocks and piling females. A comparison was not made in *L. lignorum* in this and subsequent analyses, because very few members of this species could be found in piling samples during this part of the work. There seems to be a clear specific difference in brood size with the largest broods in *L. lignorum* followed, in test block samples, by *L. quadripunctata* and *L. tripunctata*. In piling samples, the average brood size of *L. tripunctata* was nearly twice that of *L. quadripunctata*.

Composition of the Broods

A total number of 768 gravid females were examined for the developmental stages of their broods. Of these 35.4% were carrying eggs, 25% were carrying embryos, 15.8% were carrying larvae and 23.8% contained more than one type of developmental stage. The presence of mixed broods is a phenomenon not previously recorded in the literature except by Coker (1923) who found a single brood containing 6 eggs and one larva. It appears, however, that they are of frequent occurrence in all three species and widely separated stages were found in the same brood.

In the broods examined, the percentage of eggs was generally higher than of embryos which in turn was greater than the percentage of larvae. Altogether, 7,029 young stages were examined, containing 51.8% eggs, 33.9% embryos, and 14.3% larvae. This probably results from different development times for the three stages. A less likely explanation is differential mortality.

The seasonal fluctuations in the composition of the broods is shown in Figure 8. The percentage of eggs in the total stages was constant at about the 40% level for most of the year, but there was a marked increase in January and February. By May, the percentage of eggs had fallen nearly to the previous level. An interpretation of

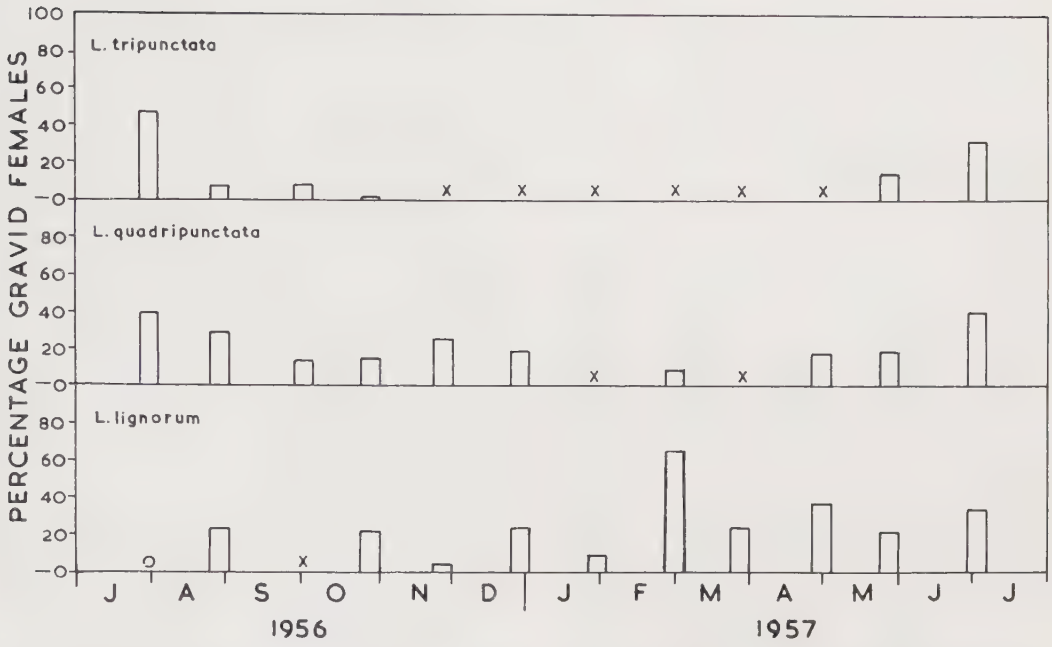

FIG. 6. The reproduction of the three species of *Limnoria* in the test blocks at Calshot (Site C) for the year July 1956 to July 1957. Each column represents the gravidity as calculated from subsamples of *Limnoria* extracted from the test blocks. O — indicates that no individuals were found in the sample; X — indicates that no gravid females were found in the sample.

these observations is that the incubation period was greatly extended during the cold winter months. The small percentage of larvae in the May sample may mean that development of the over-wintering eggs had not yet reached this stage, or that development had been completed and the young had left the pouch. The latter is more probable, for some development of the over-wintering eggs seems to have taken place in a small sample collected during March. It is probable that the first broods of the year did not leave the brood pouch until June, although the eggs deposited the previous winter did so earlier, probably between March and April.

Variation in Brood Size with Body Size

The brood sizes in gravid females of the three species have been compared with pleotelson width. Increase in brood size with body size is significant only in the case of *L. quadripunctata* ($P < 0.001$, 187 specimens). The relationship is not significant in *L. tripunctata* ($P = 0.05 - 0.1$, 71 specimens) and still less so in *L. lignorum* ($P > 0.1$, 83 specimens).

Seasonal Variation in Brood Size

The average brood size each month was calculated from the data used in the analysis of the brood composition. The monthly figures fluctuated somewhat and a better

TABLE 5. *The average brood size in gravid females of* Limnoria *from piling and test block samples*

	L. tripunctata		*L. quadripunctata*		*L. lignorum*		Mixed species	
	Piling	Test block	Piling	Test block	Piling	Test block	Piling	Test block
No. of females examined	56	70	72	168	2	84	100	367
No. of young stages	472	626	342	1,910	22	1,202	506	3,641
Average no. per female	8.4	8.9	4.7	11.4	–	14.3	5.1	9.9
Range	1–25	2–21	1–15	2–33	–	2–34	1–13	1–37

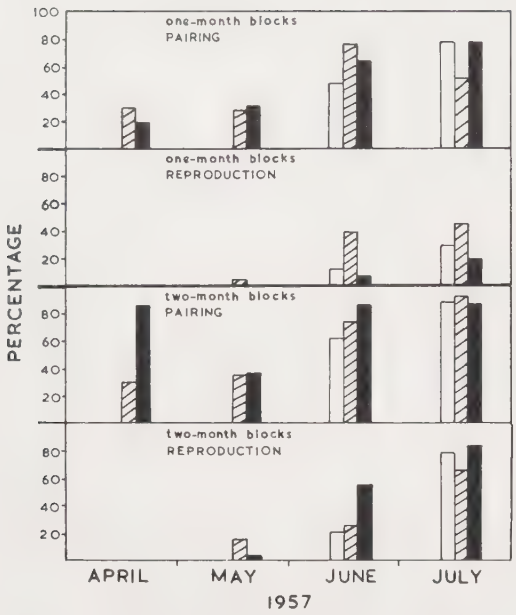

FIG. 7. Pairing and reproduction in the three species of *Limnoria* in test blocks at Southampton (Site A) for the months April to July 1957. Unfilled columns—*L. tripunctata*; hatched columns—*L. quadripunctata*; filled columns—*L. lignorum*.

idea of seasonal changes was obtained by grouping the results into three-month seasons with the first three months as winter. The only noticeable seasonal difference was seen in *L. lignorum* in which the spring broods (15.5 stages per female) were larger than the autumn broods (12.1 stages per female). A *t*-test shows this difference to be significant ($P = 0.05$) but the 95% confidence limits for the means overlap and little reliance can be placed on the significance noted.

Comparison of the Size of Gravid Females from Test Blocks and Piling

The mean pleotelson width of 110 gravid *L. tripunctata* from test blocks was 0.75 mm, a figure very close to the corresponding mean (0.74 mm) for 79 females from piling. In *L. quadripunctata*, however, the gravid females in test blocks were larger than those in piling. The mean pleotelson width of 182 test block specimens was 0.89 mm ($\sigma = 0.075$), significantly higher than the mean for 100 individuals from piling (0.84 mm, $\sigma = 0.057$).

Sex Distribution in Migrant Populations

The sex of the first migrants, those found singly in short burrows, to arrive on the test block at Site A was determined during the 1957 migratory season. In all species more males than females were found alone in the burrows. This was most marked in *L. lignorum* (208 males to 16 females) while in *L. tripunctata* it was hardly apparent (17 males to 9 females). The preponderance of males of *L. quadripunctata* was noticeable only in the block exposed during the month of maximum migration when 34 males and 10 females were found. At other times the sex ratio was 1 : 1 (41 specimens of each sex).

The total sex ratio of all *Limnoria* extracted from the Southampton test blocks was determined to see whether migration was more common in the males, as might be concluded from the previous analyses. It was found that the number of the sexes was approximately equal in *L. tripunctata* (69 males, 68 females) and in *L. quadripunctata* (239 males, 215 females) but in *L. lignorum* the ratio of males to females was approximately 2 : 1 (448 males, 242 females).

The Relative Position of the Sexes in the Burrow

During the examinations of the test blocks from Site A in 1957, the sex of the animal at the head of the burrow was noted when the burrow was occupied by a pair of *Limnoria*. A total of 436 pairs was examined and it was found that the female was usually at the head of the burrow (*L. tripunctata* 7 male, 45 female; *L. quadripunctata* 16 male, 142 female; *L. lignorum* 31 male, 195 female). It was shown above that the female did not migrate first; hence the relative position of the sexes in the burrow must be of some significance, possibly in the copulatory behavior.

Size Distribution of Limnoria *from Test Block and Piling Populations*

The mean pleotelson width of 184 *L. tripunctata* from piling and of 159 from test blocks were 0.71 mm and 0.69 mm respec-

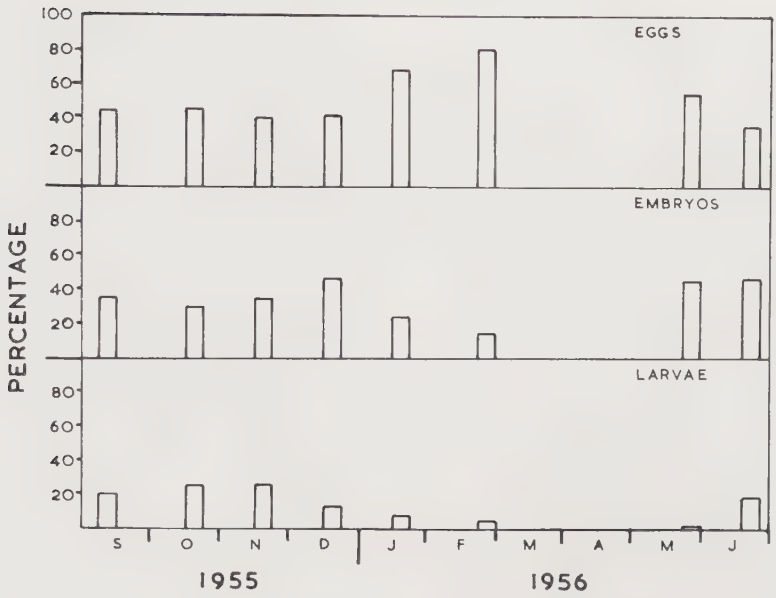

FIG. 8. The percentage composition of the broods of gravid *Limnoria* from test blocks at Calshot (Site C) between September 1955 and July 1956.

tively. These means were not significantly different. In *L. quadripunctata*, however, there was a notable size difference which a *t*-test shows to be highly significant ($P < 0.001$). The mean pleotelson width of 226 specimens from piling was 0.80 mm ($\sigma = 0.107$ mm) while that of 275 individuals from test block was 0.86 mm ($\sigma = 0.081$ mm). The 95% confidence limits for the means do not overlap. An inspection of the data shows that this difference was due to the absence of small animals from the test block population and the presence of the largest *Limnoria* in test blocks only. The inference is that, in this species at least, migration was limited to sexually mature individuals.

DISCUSSION

The Influence of Temperature Upon the Migratory Season

The migration of *Limnoria* in Southampton Water was earlier in 1957 than it was in 1956. In 1957 the migratory activity remained at a maximal level for a longer period than in 1956. This was noticeable at Southampton and Marchwood but not at Calshot.

It seems probable that the time of the onset of migration was controlled by the water temperature. The temperature conditions over the periods during which migratory activity began were about the same each year except at Calshot in 1956 where the season began at a higher temperature. With this exception, migration commenced as the water temperature was rising through 10°C and the data from the one-month blocks at Southampton suggest that migration did not begin until this temperature was reached. It is suggested, therefore, that a temperature of about 10°C stimulates the migratory instinct in *Limnoria* and the earlier migration in 1957 is attributed to the fact that this temperature was reached earlier in the year.

The prolonged migratory activity at Southampton and Marchwood in 1957 may also be interpreted on a temperature basis for, at these sites, the water was maintained at a higher temperature than normal by the discharge of warm water from an industrial plant which began operations in 1957. The effect of the warm water did not reach Calshot and here the migratory season was not unduly prolonged. It is possible that

the exceptionally mild weather in February and March 1957 contributed to the extensive summer migration.

The migratory activity declined more rapidily than the water temperature at all sites in 1956. This phenomenon was repeated in 1957 at Calshot and probably also at Southampton and Marchwood but, at these sites, observations could not be carried on long enough to be certain.

Specific differences in migratory behavior

L. quadripunctata and *L. lignorum* began to migrate at about the same time and it therefore seems likely that the water temperature which initiates migration in these species is the same. *L. tripunctata* began to migrate about a month later when the water temperature was rising through 15°C. Further work is necessary to confirm that these temperatures are characteristic of the species. From the Southampton data, it appears that most of the prolonged summer migration was due to *L. quadripunctata* and it may be that this species only was affected by the increased summer temperatures. At Calshot, where the increased temperature effect was not apparent, the migration of this species seemed to be declining in July. The sequence of the periods of maximum migration of the species was the same at both Southampton and Calshot. All three species migrated while the temperature was rising, but, whereas the activity of *L. lignorum* declined while the temperature was still rising, the migration of *L. tripunctata* tended to follow the seasonal temperature more closely, although there were indications of a decline before the highest temperatures were reached. The migratory behavior of *L. quadripunctata* resembled that of *L. lignorum* at Calshot, but was similar to that of *L. tripunctata* at Southampton.

Overcrowding as a cause of migration

This theory, developed by Menzies (1954), is based on the different brood sizes noted in gravid females from test blocks and piling. Menzies considered that the reduced fecundity of populations in piling is a result of overcrowded conditions. Hence, a small brood size is held to be indicative of overcrowding and increasing migratory activity should be correlated with a decline in brood size. Before testing this theory it was necessary not only to confirm Menzies' observations of the brood size difference but also to examine an alternative explanation, namely that the females from test blocks were larger and for this reason carried more eggs. The results given above show that the brood size difference was found only in *L. quadripunctata*, the only species, therefore, in which brood size could be taken as an index of population pressure. Again, this was the only species in which there was a significant increase in brood size with body size and in which the average size of gravid females from test blocks was larger than those from piling. There was, therefore, some basis for the alternative theory, but there was little difference between the number of eggs in females of the body sizes found to be average for test block and piling females. It is unlikely, therefore, that a size difference in the gravid females was responsible for the brood size difference, and it may be accepted that, at least in *L. quadripunctata*, there was a genuine population difference in the brood size.

No correlation between a reduction in brood size in *L. quadripunctata* and the onset of migratory activity was established as little temporal variation in the brood size of any species was found. Overcrowding as a cause of migration, therefore, was not supported. It is, in any case, unlikely in view of the seasonal regularity of migration, for overcrowding might be expected to be an irregular phenomenon unless the increased reproduction in summer caused regular overcrowding, in which case migration should follow and not precede reproduction.

Endogenous causes of migration

A more probable theory is that a migratory instinct develops at a particular stage in the life history when the organism is competent to react to environmental stimuli which trigger off the migratory be-

havior. It appears that the development of this instinct is linked with sexual maturity and that the environmental stimulus leading to migration is the attainment of a particular value by a rising temperature. In all species the migrants were large and presumably sexually mature, but more convincing evidence is seen in the close connection between migration and reproduction. There was a sexual difference in the migration, with the males migrating first, and a greater tendency for the males of *L. lignorum* to migrate. The gravid state appears to inhibit the migratory instinct as no gravid females were found among the early migrants. Migration was soon followed by reproduction and it is suggested that the function of migration lies in the dispersal of the species to less densely populated timber where reproduction may take place. It is essentially a dispersal phase in the life history with the adults and not the young forms undergoing dispersal. In this respect, *Limnoria* can be contrasted with the wood-boring molluscs such as *Teredo*. Migration, therefore, prevents overcrowding although it is probably not a direct result of it. Reproduction is not dependent upon migration, for reproductive seasons were found in the test block populations.

The reproduction of Limnoria

The sequence in which the species came into the reproductive state was that in which they migrated, suggesting that the specific differences in reproduction were a consequence of those in migratory behavior. It is probable, however, that there was little difference in the time of onset of reproduction in *L. quadripunctata* and *L. lignorum*. The water temperature which may be correlated with increased reproduction in these two species at Calshot was 10°C in 1957, a figure very similar to that given by other workers for *L. lignorum*. Johnson (1935) gives data which show an increase in reproduction in Friday Harbor, Washington, following a water temperature rise to between 9 and 10°C, while Sømme (1940) records that an average water temperature of 9.6°C coincided with the beginning of the breeding season in Norway. Reproduction in *L. tripunctata* was not detected at Calshot until the end of May, during which month the mean temperature was 12.1°C. It cannot be concluded that this necessarily reflects a specific difference, for only two specimens of *L. tripunctata* were found in the sample taken on May 1 and it may be that a larger sample of this species would have contained some gravid females.

The gravidity reached its peak in all species at the highest water temperatures and there is no evidence of a specific difference in peak gravidity as suggested by Johnson and Menzies (1956).

It seems likely, from the analysis of the percentage composition of the broods in mixed species, that some eggs are carried over the winter in a dormant condition. The data for the separate species, although inadequate because of the paucity of gravid females, suggests that this phenomenon occurs in *L. quadripunctata* and *L. lignorum* but not in *L. tripunctata*.

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NOTES AND COMMENT

Observations on the Variation of Planktonic Photosynthesis With Light Intensity, Using Both the O_2 and C^{14} Methods

In preliminary studies of light acclimation by phytoplankton, disparities were observed between the results obtained by the light and dark bottle O_2 method and the C^{14} method.

Simultaneous measurements of photosynthesis versus light intensity (P *vs.* I) by both methods were carried out on a culture of the coccolithophore, *Syracosphaera carterae*. The culture was "shade-adapted," *i.e.*, reared at a constant low light intensity of about 0.06 langlies per minute (ly/min; 3800–7200A) and then placed in full sunlight having a 24 hr mean photosynthetic intensity of approximately 0.25 ly/min with maximum values of about 0.6 ly/min. P *vs.* I relationships were measured on the "shade-adapted" culture and at two day intervals after it was placed in full sunlight.

P *vs.* I curves for the "shade-adapted" cell are shown in Figure 1, and those after two days in full daylight in Figure 2. It will be seen that results by both O_2 and C^{14} methods indicate a substantial increase in the optimum light intensity for photosynthesis and a decrease in the severity of the decline of photosynthesis occurring at supra-optimal light intensities. Little further change in the curves occurred during a further 4 days exposure to full sunlight. Hence acclimation to the higher intensities was complete by the third day. In contrast, Ryther (1956) found no acclimation by *Dunaliella euchlora, Skeletonema costatum* and *Amphidinium klebsi* exposed to full sunlight, even after 7 days exposure.

The O_2 method is commonly supposed to measure gross photosynthesis, and the C^{14} method approximately net photosynthesis. If so, relative photosynthesic rates according to the O_2 method should exceed those found by the C^{14} method at all light intensities except at the optimum, where rates are normalized to the same value. This was observed at sub-optimal light but at supra-optimal light intensities both the relative and absolute rates of photosynthesis by the C^{14} method exceeded the O_2 results. The disparity was greatest with the "shade-adapted" cells, in which case there was a very large consumption of oxygen at high light intensities concurrent with an apparently "normal" uptake of C^{14}.

The consumption of O_2 at high intensities may be attributed to photoxidation, a light driven oxidation of cellular substrate accompanied by pigment bleaching (Myers

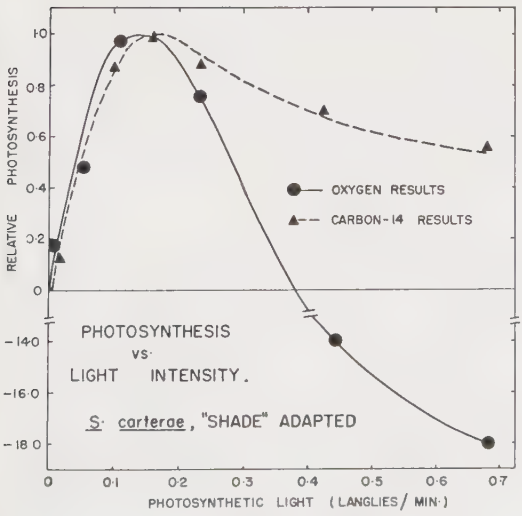

Fig. 1

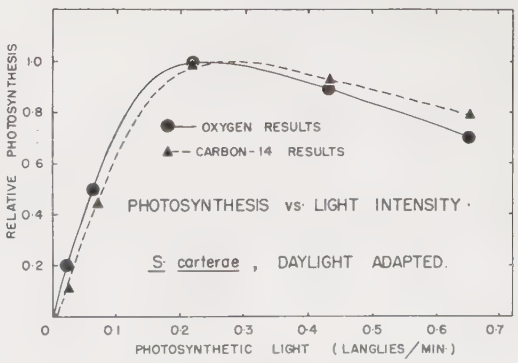

Fig. 2

and Burr 1940; Sironval and Kandler 1958). The disparity between the O_2 and the C^{14} results with the shade adapted cells in strong light could occur if photosynthesis and photoxidation were separate processes capable of acting simultaneously, as suggested by Myers and Burr (1940). If so, the C^{14} technique would not give results comparable to those from the O_2 method unless the cells were fully labeled with C^{14}. Hence, in experiments of the duration used here (approximately 4 hr) results from the tracer method would be little affected by photoxidation. The supra-optimal decrease in photosynthesis observed with the C^{14} method may be mostly attributed to photo-inhibition, a process which does not necessarily require the presence of oxygen (Avron 1960).

It seems that with "shade-adapted" cells placed in strong light, the C^{14} method measures only the approximate net rate of carbon *incorporation*, while the O_2 method measures the net change in carbon content due to the opposing processes of both gross photosynthesis and photoxidation. This suggests that the C^{14} technique would be preferable in studies of the effects of light intensity on photosynthetic carbon *uptake* by "shade-adapted" cells, but that the O_2 method would be better for productivity studies in which net changes in the amount of primary food matter are sought.

The fact that absolute C^{14} rates appear to exceed absolute O_2 rates at high light intensities, even after acclimation, suggests that some photoxidation may take place during the normal course of daily photosynthesis in phytoplankton exposed to supra-optimal light for part of the day. The observation of a midday decrease in chlorophyll content of surface plankton by Yentsch and Ryther (1957) may support this view. However, the observed ratios of O_2 to C^{14} rates (absolute) in strong light after acclimation could also result if the choice of photosynthetic quotient (1.2) used in calculating rates from O_2 results was wrong, or if this quotient varied with light intensity.

The data obtained here are insufficient to resolve the difference in results obtained by the two methods. However, it seems reasonably certain that results obtained using the C^{14} method with "shade" plankton in strong light will be overestimated and that use of either method in studies of primary aquatic production requires constant re-assessment.

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Natural Coloring Matter as an Indicator of Inshore Water Masses[1]

The discoloration of waters by natural pigments has been observed in bodies of fresh water (Birge and Juday 1934) and in coastal waters from the poles to the tropics (Emery and Stevenson 1957). Tea colored water is particularly noticeable in the lakes and estuaries of the North Gulf Coast region of Florida.

During a hydrographic survey of Ochlockonee Bay, Florida, it was discovered that the water was colored brown to the extent that absorption at 470 mμ could be measured with a Klett-Sommerson photo-

[1] Florida State University Oceanographic Institute Contribution No. 162.

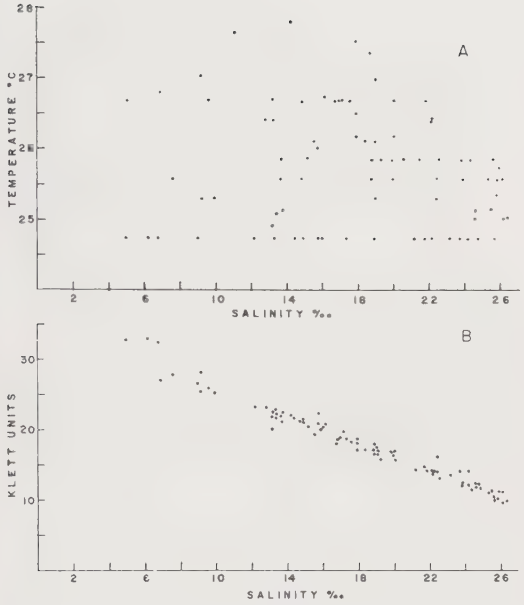

FIG. 1. Temperature-salinity diagram (A) and photocolorimeter unit-salinity diagram (B) based on surface and bottom water samples taken in the central portion of the bay on May 6, 1959, 0758–1825 hr.

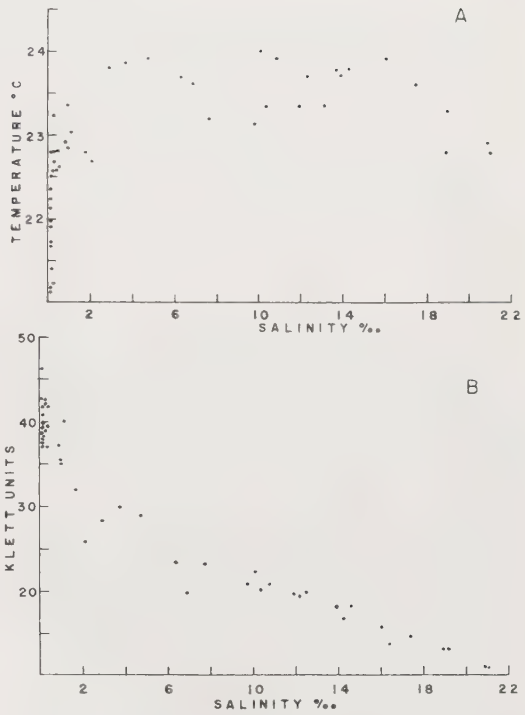

FIG. 2. Temperature–salinity diagram (A) and photocolorimeter unit–salinity diagram (B) based on surface samples taken from all regions of the bay on April 11, 1959, 1100–1630 hr.

electric colorimeter. This colored water is carried into the Ochlockonee estuary by the Sopchoppy and Ochlockonee Rivers which enter from the west. The stained river water mixes in the shallow rectangular bay with relatively unstained water from the Gulf of Mexico which lies to the east.

On May 6, 1959, a survey was completed which involved obtaining surface and bottom hydrographic data from 6 stations in the central portion of the Bay at regular time intervals over a 12-hr period. Figure 1A is a temperature-salinity diagram based on the data of this survey. This diagram is difficult to interpret because of the degree of scatter and shows the effects, in part, of solar insolation progressively increasing during the day. Figure 1B is a plot of salinity *vs.* Klett readings obtained from filtered portions of the above water samples. This plot indicates that 1) two water masses are mixing in this region of the bay and 2) dye concentration varies inversely with the amount of Gulf water present.

The survey of April 11, 1959, involved collecting surface water samples from the entire estuary. As on May 6 these data indicate a closer identifying relationship between color and salinity (Fig. 2B) than between temperature and salinity (Fig. 2A). The inverse relationship betwen salinity and dye concentration was illustrated again on May 8, 1959, when a 25-station grid was established to trace the path of the water leaving the Ochlockonee estuary in the ebb tide.

The relationship between color and salinity is less linear on some days than on others presumably because the fresh water entering the estuary is not always stained to the same degree. This means that to follow a water mass by measuring color alone it is necessary to establish a salinity-color curve for each survey. However, since readings on several hundred samples can be obtained in a few hours, water coloration may be used practically in inshore areas to identify water masses and to trace their movements. This coloration is present the year around

along the North Gulf Coast and is present seasonally in other coastal waters.

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NOTES ON THE CHLOROPHYLL METHOD OF ESTIMATING THE PHOTOSYNTHETIC CAPACITY OF STREAM PERIPHYTON[1]

Considerable interest has been shown in the use of chlorophyll data to estimate the photosynthetic capacity of aquatic ecosystems. Although this method is still in a developmental stage, it has been demonstrated in at least an approximate fashion that the rate of photosynthesis is a function of chlorophyll *a* content (Ryther 1956; Odum 1959; Strickland 1960). The method appears to have particular significance in streams because of the difficulty in using either the light-and-dark bottle or C^{14} method in flowing waters; both require containment of water in a bottle. McConnell and Sigler (1959) have reported the estimation of periphyton chlorophyll on artificial and natural rock substrates in Logan River, Utah. Grzenda and Brehmer (1960) have measured the chlorophyll content of artificial substrates in a Michigan stream and refer also to unpublished work by F. F. Hooper, R. C. Ball, and D. W. Hayne.

When placed on the stream bottom, an artificial substrate is an empty habitat subject to colonization. The chlorophyll content and the photosynthetic capacity measured on an artificial substrate may thus be a function of the age of the periphyton population as well as a function of the environment. Such developing populations may not reflect the same relationship to external factors as those on a natural substrate that has been in the stream for a long time.

Three experiments were carried out in Valley Creek, Washington County, Minnesota, to clarify this point. The substrates used throughout were concrete cylinders 5.1 cm in diameter and 7.6 cm long. They were placed on their sides in open sunshine, in riffle locations where the native rock size was similar to that of the artificial substrates, where the water was about 15 cm deep, and where the surface water velocity was about 1 m/sec. On removal from the stream, each substrate was placed into a jar with sufficient 90% acetone to cover it; the jar was stoppered, wrapped in aluminum foil, and stored temporarily in a portable ice chest with wet ice. After about 24

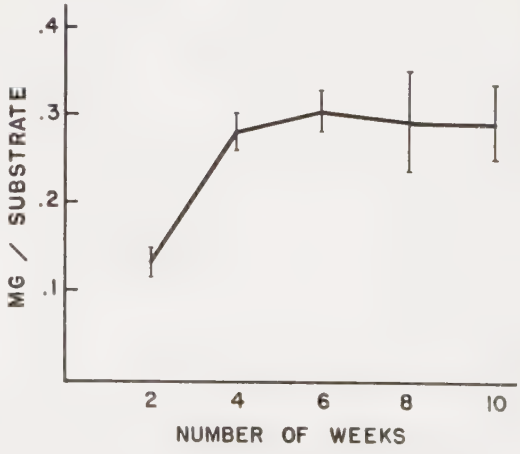

FIG. 1. Chlorophyll *a* on artificial substrates at different numbers of weeks following placement in stream, summer of 1958. Vertical lines represent two standard errors on each side of the mean.

[1] Paper No. 4527 Scientific Journal Series, Minnesota Agricultural Experiment Station. The investigations were supported in part by a grant from the Graduate School of the University of Minnesota. Assistance in the 1958 field studies was received from David G. Savoie. The assistance of Robert J. Knapp in both field and laboratory is gratefully acknowledged.

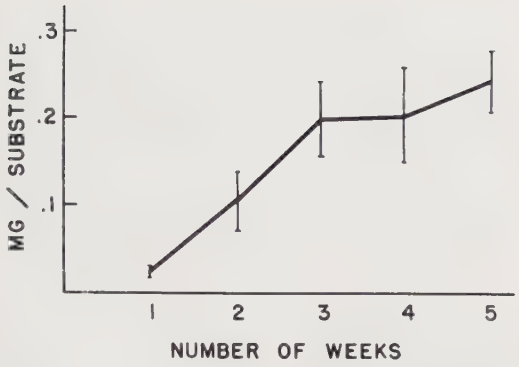

FIG. 2. Chlorophyll *a* on artificial substrates at different numbers of weeks following placement in stream, autumn of 1958. Vertical lines represent two standard errors on each side of the mean.

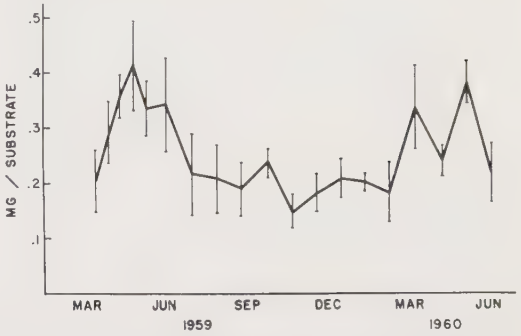

FIG. 3. Chlorophyll *a* on artificial substrates during 1959–1960. Substrates were placed in stream on March 13, 1959. Vertical lines represent two standard errors on each side of the mean.

hr, the pigment–acetone solution was filtered through qualitative filter paper and brought up to 50 ml with additional 90% acetone. From this point, chlorophyll *a* was determined by the method of Richards and Thompson (1952) using a Beckman DU spectrophotometer.

On July 8, 1958, 40 substrates were placed in the stream. Five times, at two-week intervals, 8 substrates were removed and analyzed. Chlorophyll *a* per substrate increased to about 0.15 mg in 2 weeks, to about 0.3 mg in 4 weeks, and, apparently reaching saturation at that time, remained at about 0.3 mg for the remainder of the period (Fig. 1).

The second experiment was conducted during the autumn instead of the summer, the time interval was 1 week instead of 2, and the substrates were placed in the stream in reverse order; that is, 4 substrates were placed in the stream on September 16, 1958, and 4 more were placed in the same area on each of 4 additional dates at 1-week intervals. All were then removed on the same date, October 21. Chlorophyll *a* content per substrate was about 0.025 mg at 1 week, about 0.1 mg at 2 weeks, and about 0.2 mg at 3 weeks, after which there was an apparent tendency for it to remain constant (Fig. 2).

Since there appeared to be seasonal influence, the third experiment was conducted over a period of an entire year. On March 13, 1959, about 120 substrates were placed in the stream. They were removed and analyzed for chlorophyll *a* every 2 weeks for 5 dates, and thereafter once every 4 weeks. The number of substrates removed was 4 on each of the first 5 dates and 6 thereafter. This program was continued until June 17, 1960.

As expected from the previous experiments, chlorophyll *a* increased rapidly during the first few weeks (Fig. 3). A maximum mean of about 0.4 mg per substrate occurred on May 9, 1959, 8 weeks after placement. Subsequently, chlorophyll *a* decreased to reach a minimum during November. Winter values were low, but increases occurred during the following spring. A temporary drop occurred in late April, possibly due to erosion by high waters. This was followed by a rapid recovery and a drop in early summer, suggesting the return to normal summer low conditions.

In all three experiments, there was a certain length of time immediately after placement of artificial substrates during which chlorophyll content increased toward a maximum, after which some level of population saturation apparently was reached. Subsequently, the level of chlorophyll appeared to vary seasonally and was then no longer related to the length of time that the substrate had been in the water. The time for chlorophyll *a* to reach a maximum varied in Valley Creek from about 3 to 8 weeks, apparently depending on environ-

mental conditions. Thus, it would appear that substrates removed and analyzed during this initial period of time would result in underestimates of the photosynthetic capacity of a stream's periphyton.

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A NEW RECORD FOR *BALANUS BALANOIDES* (L.)

With regard to the broad geographical distribution of *Balanus balanoides* (L.) on the Atlantic coast of America, Barnes (1958) asserted that the southern limit for this cirripede of boreo-arctic regions may be set at 38°N latitude. More recently Wells, Wells and Gray (1960) collected specimens from the region of Cape Hatteras.

During the period March 23 to 25, 1961, I collected 26 individuals of *B. balanoides* from Town Marsh and Fort Macon, Beaufort, North Carolina. At that time sea-surface temperatures had attained 14.6°C and salinity was in the neighborhood of 33.3‰.

The specimens had basal diameters ranging from 3 to 13 mm. Cirral beat was normal at a temperature of 15° to 17°C. Barnes (1958) remarked that general metabolic activities and growth of *B. balanoides* under natural conditions do not appear to be impaired at a temperature as high as 20°C. While the paucity of this species in the Beaufort area did not permit reliable field observations on its resistance to changes in temperature, in the laboratory 25°C seemed to be lethal. Apparently water temperatures in Beaufort do not reach lethal limits for *B. balanoides* cyprids during the colder months. This accords well with Dr. I. E. Gray's suggestion (*personal communication*) that a number of cold-water forms may slip around Cape Hatteras under favorable conditions in the winter and take up temporary residence farther south. Evidence for this is demonstrated by the distribution of the mussel *Mytilus edulis* on the Carolina coast (Wells and Gray 1960).

Twelve specimens have been deposited at the U. S. National Museum, Washington, D.C., under catalogue number USNM 107163.

ACKNOWLEDGMENTS

I am greatly indebted to Dr. Dora Priaulx Henry for verifying my identification, and to Drs C. G. Bookhout, J. D. Costlow, Jr., and I. E. Gray for their guidance. The facilities accorded by the Duke Marine Laboratory are deeply appreciated.

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A Plankton Sub-sampler[1]

There is a need in plankton analysis methods for a sub-sampling device which will permit division of samples for independent analyses.

The plankton sub-sampler described in this note is a plexiglass modification of one designed by Dr. Raymond P. Langford of the University of Toronto. It consists of two ⅛ in. thick plexiglass tubes, one of which fits snugly into the other and is divided into 4 sections (Fig. 1). The outer cylinder has an outside diameter of 1¾ in., an inside diameter of 1½ in. and is 5 in. long. The corresponding dimensions of the inner cylinder are 1½, 1¼ and 6 in. A plexiglass base, ⅛ in. thick and 1¾ in. in diameter, is glued to one end of the outer tube and a circular piece of flat, soft rubber glued to the base to ensure a watertight seal between the base and the inner cylinder and its partitions. A ¼-in. diameter hole extends through the base and rubber. Three small legs are glued to the base to permit standing the sub-sampler when the drainage hole is stoppered. The inner cylinder is partitioned by two pieces of 6 × 1¼ × 1/16 in. plexiglass, slotted, fitted and cemented in place at right angles to each other. Special care should be taken to completely seal all joints. The inner cylinder is longer than the outer one to permit ease of removal. Dr. Langford's model was constructed of two brass cylinders, the inner one divided into 8 sections and the entire device measuring approximately 10 in. high by 4 in. in diameter.

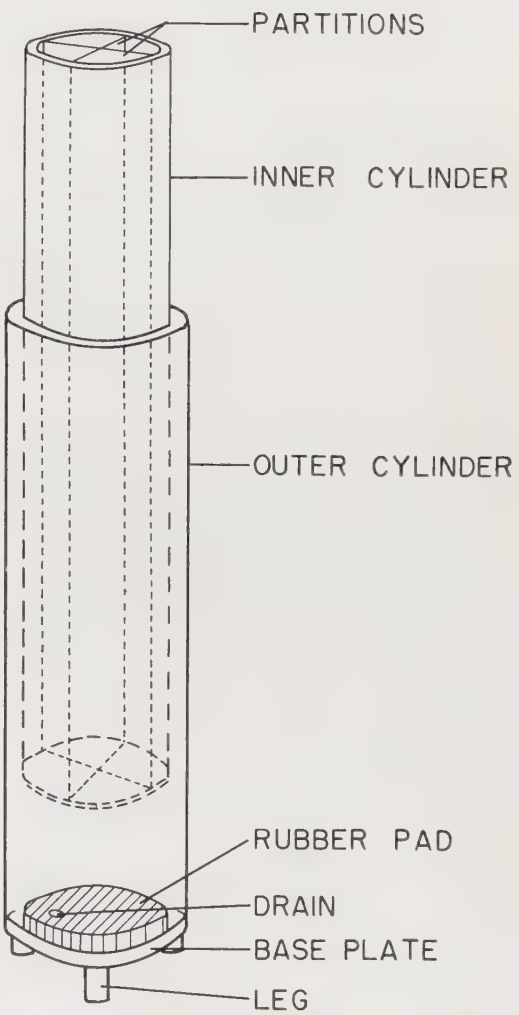

Fig. 1. Plankton sub-sampler.

In use, the inner cylinder is removed, the hole stoppered with a small rubber cork and the plankton sample poured into the outer cylinder. The inner cylinder is then inserted until its lower edge is just above the surface of the sample. The sample is then agitated until homogenous and the inner cylinder immediately pushed down firmly onto the rubber base so that one of the four sections encompasses the drain hole. It is safer to rest the outer cylinder on a table when this is done, to prevent unnecessary strain on the bond between the cylinder and the base plate. While holding the sub-sampler over a vial, the plug is removed with a pair of forceps and one-fourth of the original sample removed. Flushing the empty section with distilled water from a polyethylene "squeeze" bottle ensures complete removal of all organisms. The remaining three-fourths of the original sample can be returned to the original container after removing the inner cylinder.

In practice, it was found that an original sample of 60 ml worked very well. The size of this sample, however, can be regulated according to the nature of the sample. A

[1] Supported by N.S.F. Grant G-12428.

larger initial dilution was used when dividing drift samples which contained a heterogenous mixture of plankton, insects and their remains, detritus, and fragments of vegetation. In the case of samples containing an extremely high concentration of organisms, the fraction removed can be again sub-sampled until it is workable.

It is to be expected that a device of this sort is subject to the errors inherent in any method of dividing samples, rare species may be missed and accuracy decreases as numbers decrease, but it has proven useful and time-saving. A series of counts performed upon a sample of organisms ranging in size from *Ceratium* to amphipods gave quite satisfactory results. In this test, organisms in all four chambers were counted and compared. The sub-sampler also has the advantage of being inexpensive and simple to construct.

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Comment on "Water Transport in Lake Huron"

Powers and Ayers (1960) have made a bold estimate of the "inflow into Lake Huron from its own watershed."

From a qualitative bucket drift and only two drogue measurements they calculated the transport of water from Lake Michigan into Lake Huron for August 6, 1957. This they have combined with *monthly* flows of the St. Marys and St. Clair Rivers of August of the *previous* year to provide an estimate of the watershed yield.

It does not seem necessary to combine data from two separate years. Therefore, repeating the calculation using the Lake Michigan outflow given by Powers and Ayers, and monthly means for August 1957 provided by U. S. Lake Survey (personal communication) we obtain the following: there is the St. Clair River outflow of 185,000 cfs from which is subtracted the Lake Michigan inflow of 55,000 cfs and the St. Marys River inflow of 64,000 cfs, leaving the Lake Huron contribution at 66,000 cfs.

Fortuitously, this value agrees more closely with Powers and Ayers' alternate calculation of 67,000 cfs (obtained by combining Horton and Grunsky's (1927) estimate of mean summer flows of St. Clair River and St. Marys River with Judson's (1909) estimate of Lake Michigan outflow) than the 71,000 cfs they obtained by mixing 1956 and 1957 data, though the use of a single day's flow measurement with monthly means at the same period also seems very difficult to justify.

These estimates were calculated from the difference between the St. Clair River outflow and the sum of inflow from Lake Superior (St. Marys River) and Lake Michigan. An equation expressing a full water balance equation for the lake is the following,

$$(R - \Delta S + P - E - G) = (O - I) \quad \ldots \quad (1)$$

where ΔS is the change in storage, P is the precipitation on to the lake, I is the river inflow from other watersheds, R is the runoff from its own watershed, E is the evaporation from the lake, G is the groundwater seepage from the lake and O is the river outflow. It is clear that "watershed inflow" calculated above includes all the terms on the left-hand side of equation (1). The use of a single day's measurement of Lake Michigan outflow with long-term averages of river flows, implies that all changes in single terms on the left-hand side of equation (1) are compensated for within that side of the equation, and that there is negligible dependence between terms on either side of the equation. This has not been demonstrated.

In conclusion, it is felt that the combining of daily and monthly flows has not been justified and that the combining of data from two different years is unnecessary. The quantity calculated, $R - \Delta S + P - E - G$, will of course be more meaningful when its component parts are separated. These calculations do illustrate, however, the importance of monitoring the outflow

from Lake Michigan and to do such continuously if possible.

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We have studied Keith Rodgers' comments. We find them to be somewhat trivial.

The chance to estimate the contribution of the Lake Huron watershed was an unexpected by-product of another study. In making the estimate it was necessary to use such data as were available.

If it be boldness to use data of limited strength in making the first approximation of a parameter previously unestimable, then we were bold. Indeed we must have been *doubly* bold, for we made *two* estimates from two completely independent sets of limited-strength data and obtained reasonable agreement.

Keith, also boldly, combines daily flow with monthly flows (though he claims this is unjustified) to produce a third estimate slightly stronger than ours, but still weak. His estimate agrees well with our two.

Keith calls the agreement of his estimate with ours "fortuitous." We suggest that it might be at least partially confirmatory to the assumption underlying our "mixing" of 1956 and 1957 data: that Lake Huron in the Augusts of two reasonably comparable years and under two comparable wind regimens might be expected to behave in about the same way in both years.

It seems to us that Keith's failure to obtain an estimate greatly different from ours should lead him to the suspicion that, in at least some years, there *might be* internal compensation in the left-hand side of his equation.

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Style Manual for Biological Journals

Prepared by:

The Committee on Form and Style of the Conference of Biological Editors'

The *Style Manual* is designed for biologists preparing manuscripts for publication in scientific journals. Style is interpreted broadly to mean forms of expression in scholarly writing, and the general technical requirements of journals, such as details for typing manuscripts, standard abbreviations, and citation of references. The instructions and recommendations represent good usage and practice.

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